

# MINUTES OF THE 47th GENERAL ASSEMBLY OF THE EUROPEAN ASSOCIATION FOR THE STUDY OF DIABETES

held in Lisbon International Fair, Portugal, Thursday 15 September 2011 at 18:00

Present:	Dr. U. Smith	(President)
	Dr. A.J.M. Boulton	(Vice President)
	Dr. F. Bosch	(Vice President)
	Dr. G. Spinas	(Honorary Treasurer)
	Dr. M. Walker	(Honorary Secretary)
	Dr. J. Zierath	(Editor-in-Chief, Diabetologia)
	Dr. C. J. Tack	(Chair, PGEC)
	Dr. V. Jörgens	(Executive Director)
	Dr. M. Grüsser	(Vice Director)
	and 47 members	

The President, Dr. Smith, welcomed everyone to the 47th General Assembly.

## 1. MINUTES 46th GENERAL ASSEMBLY 2010

Since there were no comments, the minutes were approved unanimously and officially signed as a correct record.

## 2. REPORTS

### a) President

The President's report to the members on the activities of EASD was given in the President's Address before the Minkowski Lecture. It is available under:  
<http://easd.conference2web.com/content/1029>

The President reported on the various activities and expressed his thanks to all partners. The postgraduate courses continued to be successful and attracted many young researchers. Dr. Smith reported that as expected the EASD Annual Meeting this year in Lisbon is doing very well and the number of delegates attending has increased. Dr. Smith thanked all members of the EASD Office and the Executive Committee, for their commitment and hard work.

### b) Honorary Treasurer

Dr. Spinas reported that EASD is doing very well financially. The income had increased in 2010 by approx. one million Euros from membership, registration fees and earnings from the Stockholm Annual Meeting. He said the administrative cost of 2.15% is very low. The expenditure had increased in 2010 because funds had been made to the Foundation.

Dr. Spinas expressed his thanks to the Executive Committee and to Drs. Grüsser and Jörgens for their support and advice and to the dedicated professional team in Düsseldorf. Special thanks were given to Mrs. Klee, Mrs. Deparade-Oertel and Ms. Weiss for the precise handling of the accounts. As his term of office was coming to an end, Dr. Spinas said farewell and expressed that it has been a very rewarding time and a tremendous privilege for him to serve as Honorary Treasurer and that EASD was living up to its mission.

The President thanked the Honorary Treasurer and on behalf of the Executive Committee for his diligence and asked if there were any questions. There were no further comments.

**c) Honorary Auditors**

The President asked the Honorary Auditor, Dr. Roden for his report. Dr. Roden confirmed that the accounts had been checked carefully and were in perfect order. Dr. Smith asked for the vote to accept the accounts.

The Honorary Treasurer was unanimously discharged (38 votes for and 1 abstention).

**d) Honorary Secretary**

Dr. Walker reported that 2145 abstracts had been submitted; of the 1352 which were accepted 264 were orals. The top three countries for abstract submission were USA, UK and Germany.

Dr. Walker also reported that there had been positive response to the App.

Dr. Walker closed his report by thanking all members of the EASD staff, in particular Ms. H. Goliberzuch and Mrs. M. Toledo, for their outstanding help and support with the organisation of the EASD Annual Meetings.

Dr. Smith thanked Dr. Walker for his diligence and asked if there were any questions. There were no further comments.

**e) Editor-in-Chief, Diabetologia**

Dr. Zierath briefly reported that the impact factor of Diabetologia was the highest ever and the journal continued to be successful. She also expressed her thanks to the team in Bristol and the outgoing associate editors Drs. Bierhaus and Beguinot.

Dr. Smith thanked Dr. Zierath for her enthusiasm and dedication to the journal.

**f) Chair, Postgraduate Education Committee**

Dr. Tack also reported that the web education sessions were progressing very well. Dr. Tack thanked Brian Carey, Leona Pecirep and Mary Hata for their friendly assistance.

Dr. Smith thanked Dr. Tack for his diligence in his role as Chair of the Postgraduate Education Committee.

**g) Chair, Extra-European Postgraduate Activities**

Dr. Boulton reported on the successful three courses: Nepal in November 2010, China in December 2010 and Best of EASD-India Course held in Cochin and Bangalore in February 2011 had all been very well attended. Dr. Boulton reported that courses were being planned in

China and South Africa. Dr. Boulton expressed his thanks to the staff of EASD in particular Mary Hata; he also thanked the sponsors and the collaboration with ADA and IDF.

Dr. Smith thanked Dr. Boulton for his hard work in organising the extra-European activities.

**3. ELECTIONS****President (2011–2014)**

The General Assembly unanimously approved the election of Dr. A.J.M. Boulton with 45 votes and 1 abstention.

**Vice President (2011 – 2014)**

The election of Dr. S. Del Prato was unanimously approved with 46 votes and 1 abstention.

**Honorary Treasurer (2011 – 2014)**

The election of Dr. M. Roden was unanimously approved with 46 votes and 1 abstention.

**Council Members (2012 - 2015)**

The election of Drs. H. Beck-Nielsen, S. Bolgarskaya, A. Novials Sarda and R. Weitgasser was unanimously approved with 44 votes and 3 abstentions.

**Honorary Auditor (2011 - 2014)**

The election of Drs. P. Diem and L. Gardete-Correia was unanimously approved with 46 votes and 1 abstention.

#### **4. STUDY GROUPS**

Dr. Boulton reported that the EASD Study Groups and representatives had been invited to a forum during the Annual Meeting. At the forum he noted that presently, the format of constitution varies greatly across the study group. Dr. Boulton also suggested that study group members would be ideal candidates for EFSD grant reviewers and encouraged all study groups in listing the names of potential reviewers.

#### **5. HONORARY MEMBERSHIP**

The nomination for Dr. C. Wollheim was unanimously approved. Dr. Smith thanked Dr. C. Wollheim for his outstanding contribution to diabetes research.

#### **6. ANY OTHER BUSINESS**

Dr. Wim Wientjens thanked EASD for their marvellous work. He also commented the possibilities if future IDF meetings would eventually be allowed to take place in the EU. Dr. Pierre Lefèbvre suggested that talks between the EASD Executive Committee and the IDF board should take place.

Dr. Smith thanked Dr. Wientjens and Dr. Lefèbvre for their comments and assured them that EASD and IDF are continuously in correspondence with each other. Dr. Smith thanked the industry for their support. He also expressed his sincere gratitude to the Local Organising Committee for their outstanding contribution to the organisation of the 47th EASD Annual Meeting. The President warmly thanked Dr. Jörgens and the EASD team in Düsseldorf for their dedicated work.

Dr. Smith brought the General Assembly to a close at 18:50.

# Agenda for the 48th General Assembly of the European Association for the Study of Diabetes

to be held in the Rubner Hall, Messe Berlin, Germany  
on Thursday 4 October 2012 at 18:00

## 1. Minutes of the 47th General Assembly, Lisbon, Portugal 2011

## 2. Reports

- |  |                       |
|--|-----------------------|
| a) President   | A.J.M. Boulton        |
| b) Honorary Treasurer  | M. Roden              |
| c) Honorary Auditors   | P. Diem               |
|  | L. M. Gardete-Correia |
| d) Honorary Secretary  | M. Walker             |
| e) Editor-in-Chief, Diabetologia                                       | J. Zierath            |
| f) Chair, Postgraduate Education<br>Committee                          | C. Tack               |
| g) Chair, Extra-European Postgraduate Activities<br>and Secretary PGEC | L. Czupryniak         |

## 3. Elections

- |                                  |                      |
|----------------------------------|----------------------|
| a) Vice President (2012 – 2015)  | in place of F. Bosch |
| b) Council Members (2013 - 2016) | in place of          |
|                                  | F. Beguinot          |
|                                  | J. Dekker            |
|                                  | F. Karpe             |
|                                  | V. Urbanavicius      |

## 4. Study Groups

## 5. Honorary Membership

## 6. Any other business



# 48th EASD Annual Meeting of the European Association for the Study of Diabetes

Berlin, Germany, 1 – 5 October 2012

## Abstracts

### Index of Oral Presentations

- OP 01 Incretin based therapies
- OP 02 Impact of bariatric surgery
- OP 03 Hyperglycaemia and the brain
- OP 04 Insulin action in the liver
- OP 05 New modulators of energy expenditure
- OP 06 What's new in the treatment of diabetic nephropathy?
- OP 07 What's new in insulin therapy?
- OP 08 Diagnosing and treating diabetic neuropathy
- OP 09 The effects of interventions in reality
- OP 10 Type 2 diabetes: from risk models to survival
- OP 11 Links between obesity, inflammation and insulin resistance
- OP 12 Dynamics of beta cell signal transduction
- OP 13 Nutritional approaches to body composition and liver fat
- OP 14 Can we improve outcomes in diabetic pregnancy?
- OP 15 Role of the immune system in type 1 diabetes
- OP 16 Mechanisms of insulin action
- OP 17 Mitochondria: signalling power plants of the beta cell
- OP 18 The -omics frontier: applications of new technologies
- OP 19 Novel therapies
- OP 20 Insulin: beyond traditional delivery
- OP 21 Stem cells in chronic complications
- OP 22 Mitochondria and insulin action
- OP 23 Complications and biomarkers
- OP 24 The transcriptome of healthy and stressed beta cells
- OP 25 Intervention studies in type 1 diabetes
- OP 26 Causes and consequences of diabetic nephropathy
- OP 27 New pathways involved in the cross talk between immune cells and metabolic tissues
- OP 28 Type 1 diabetes mellitus: acute and chronic complications
- OP 29 Genetics of type 1 and type 2 diabetes
- OP 30 Beta cell function in vivo
- OP 31 The importance of glycaemic control: results from large scale studies
- OP 32 Insights in diabetic retinopathy
- OP 33 Devices, algorithms and their application
- OP 34 Profiling glucose and clinical trials
- OP 35 Regulators of adipose tissue expansion
- OP 36 Non-coding RNAs in the beta cells
- OP 37 Metformin actions and benefits
- OP 38 Hypoglycaemia
- OP 39 Diabetes education and its clinical impact
- OP 40 Glucose fluctuations and cardiac complications
- OP 41 Modifiers and markers for cancer in type 2 diabetes
- OP 42 Changes over time in type 1 diabetes

- OP 43 SGLT-2 inhibitors
- OP 44 Mechanisms of incretin action
- OP 45 Diabetes and depression
- OP 46 Foot ulceration: Can we do better?
- OP 47 Tuning into the rhythm of clock genes in diabetes
- OP 48 Imaging beta cell mass in vivo

### Index of Poster Sessions

- PS 001 Autoimmune diabetes
- PS 002 Genetics of type 1 diabetes
- PS 003 Genetics of type 2 diabetes
- PS 004 "Omics" in type 2 diabetes
- PS 005 Risk factors for type 2 diabetes
- PS 006 Lifestyle factors and diabetes
- PS 007 Environmental factors in type 1 diabetes
- PS 008 Monogenic forms of diabetes
- PS 009 Diabetes, cardiovascular disease and mortality
- PS 010 Burden of diabetes and approaches to decrease it
- PS 011 Characterisation of patients with type 2 diabetes
- PS 012 Diagnosis of type 2 diabetes
- PS 013 Risk scores for type 2 diabetes and disease complications
- PS 014 Beta cell GPCRs and other signalling mechanisms
- PS 015 Beta cell receptor tyrosine kinase and other signalling mechanisms
- PS 016 Secretory granule and cytoskeleton dynamics in the beta cell
- PS 017 Insulin secretion and exocytosis: novel aspects
- PS 018 Islet cell development and generation I
- PS 019 Islet cell development and generation II
- PS 020 Islet transplantation
- PS 021 Experimental immunology and models of type 1 diabetes
- PS 022 Intervention in type 1 diabetes
- PS 023 Functional beta cell mass in type 1 and type 2 diabetes
- PS 024 Immune and viral correlates of beta cell failure
- PS 025 Clinical immunology: immune markers in type 1 diabetes
- PS 026 Beta cell channels
- PS 027 Cytokines and beta cell damage
- PS 028 Models of beta cell failure in type 2 diabetes
- PS 029 Beta cell lipotoxicity
- PS 030 Beta cell glucotoxicity and oxidative stress
- PS 031 Beta cell apoptosis and survival
- PS 032 In vitro insulin action
- PS 033 In vivo insulin action in animals
- PS 034 In vivo insulin action in humans

- |   |   |
|---|---|
| PS 035 Non-pancreatic molecules and glucose metabolism          | PS 077 Insulin therapy: clinical perspectives                                       |
| PS 036 Incretins  | PS 078 Psychological aspects I  |
| PS 037 Gastric bypass surgery                                   | PS 079 Psychological aspects II   |
| PS 038 Mitochondria   | PS 080 Psychological aspects: stress and depression                                 |
| PS 039 Exercise: impact and benefits                            | PS 081 Clinical diabetes care   |
| PS 040 Aspects of carbohydrate metabolism                       | PS 082 Diabetes education   |
| PS 041 Hypoglycaemia: relation to therapy                       | PS 083 Diabetes care in different settings  |
| PS 042 Hypoglycaemia: mechanisms and effects                    | PS 084 Learning from the global pandemic  |
| PS 043 Regulation of fatty acid metabolism                      | PS 085 Cost and quality of diabetes care  |
| PS 044 Mechanisms of NAFLD progression                          | PS 086 PumPS and new devices  |
| PS 045 Novel players in the development of insulin resistance   | PS 087 Blood glucose self monitoring  |
| PS 046 Immune cells and inflammation in type 2 diabetes         | PS 088 Continuous glucose monitoring  |
| PS 047 Lipid and inflammatory signalling in skeletal muscles    | PS 089 Continuous glucose monitoring with pumps                                     |
| PS 048 Mechanisms for adipocytes differentiation                | PS 090 Predicting pregnancy outcomes  |
| PS 049 Secreted proteins and organ cross-talk                   | PS 091 Consequences of gestational diabetes   |
| PS 050 Metabolic regulation in the brain                        | PS 092 Biomarkers in gestational diabetes mellitus                                  |
| PS 051 Cardiovascular complications                             | PS 093 Diagnosis of gestational diabetes mellitus                                   |
| PS 052 Diabetes and cancer                                      | PS 094 Lessons from microvascular cohort studies                                    |
| PS 053 Glucagon-related peptides and receptors                  | PS 095 Treatment of microvascular complications                                     |
| PS 054 Beneficial effect of bariatric surgery and weight loss   | PS 096 Experimental: nephropathy and retinopathy                                    |
| PS 055 Incretin based therapies                                 | PS 097 Predicting nephropathy and retinopathy                                       |
| PS 056 SGLT-2 I   | PS 098 Diabetic nephropathy and retinopathy screening and mechanisms                |
| PS 057 SGLT-2 II  | PS 099 Predicting nephropathy   |
| PS 058 SGLT-2 III   | PS 100 Small fibres in neuropathy: clinical   |
| PS 059 SGLT-2 IV  | PS 101 Foot ulceration and amputation: treatment outcomes                           |
| PS 060 Incretins and atherosclerosis                            | PS 102 Mechanisms and treatment of diabetic neuropathy                              |
| PS 061 Oral therapies: metformin, sensitizers and sulfonylureas | PS 103 Diabetic foot: risk factors  |
| PS 062 Clinical studies and GLP-1 agonists                      | PS 104 Genes and chronic complications  |
| PS 063 GLP-1 based therapies                                    | PS 105 Epidemiology and cardiovascular complications                                |
| PS 064 Incretin related novel therapies                         | PS 106 Microvascular complications in experimental studies                          |
| PS 065 DPP-4 and other secretagogues                            | PS 107 Risk, pathophysiology and cardiovascular outcome in type 2 diabetes mellitus |
| PS 066 DPP-4 inhibitors: clinical studies                       | PS 108 Pharmacological aspects and cardiovascular outcome                           |
| PS 067 DPP-4 inhibitors I                                       | PS 109 Vascular mechanisms and biomarkers   |
| PS 068 DPP-4 inhibitors II                                      | PS 110 Effects of medication on cell culture models                                 |
| PS 069 Screening, prevention and early management               | PS 111 Macrovascular complications in experimental studies                          |
| PS 070 Nutrition, diet and diabetes                             | PS 112 Diabetes and sleep apnoea  |
| PS 071 Novel unconventional therapies                           |   |
| PS 072 Diabetes in childhood                                    |   |
| PS 073 New insulins I   |   |
| PS 074 New insulins II  |   |
| PS 075 Insulin delivery   |   |
| PS 076 Initiation of insulin therapy                            |   |

## OP 01 Incretin based therapies

1

### Liraglutide trial in paediatric subjects with type 2 diabetes: safety, tolerability and pharmacokinetics/pharmacodynamics

T. Battelino<sup>1</sup>, D. Klein<sup>2</sup>, D.J. Chatterjee<sup>3</sup>, P. Hale<sup>3</sup>, C.T. Chang<sup>3</sup>, S. Arslanian<sup>4</sup><sup>1</sup>University Medical Center, Ljubljana, Slovenia, <sup>2</sup>Cincinnati Children'sHospital Medical Center, Cincinnati, <sup>3</sup>Novo Nordisk Inc, Princeton,<sup>4</sup>University of Pittsburgh School of Medicine, USA.

**Background and aims:** Prevalence of T2D is increasing in adolescents and the need for more treatment options is escalating. We investigated the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics of liraglutide QD in adolescent subjects (10–17 years old) with T2D in a randomised, double-blind, placebo-controlled trial.

**Materials and methods:** Subjects on diet/exercise or metformin were randomised 2:1 to liraglutide (14) or placebo (7) injections. Starting at 0.3 mg, doses were escalated weekly to 0.6, 0.9, 1.2, 1.8 mg/day (or placebo equivalent) for 5 weeks.

**Results:** Nineteen subjects completed the trial. Groups were matched at baseline, with mean (SD) age 14.8 (2.2) years, weight 113.2 (35.6) kg, diabetes duration 1.7 (1.4) years and HbA<sub>1c</sub> 8.1% (1.2%). No serious adverse events (SAEs), including severe hypoglycaemia, were reported; AEs were mild in liraglutide-treated subjects and mild to moderate in placebo-treated subjects. Transient, dose- and body weight-independent gastrointestinal AEs were most common at lower doses of liraglutide (Table). There were no significant changes in other measures of safety and tolerability. No pancreatitis was reported and calcitonin levels were within the normal range in all subjects and did not increase meaningfully with liraglutide. After administration of 1.8 mg liraglutide, mean  $t_{1/2}$  was 12 h and CL/F 1.7 L/h, similar to observations in adults ( $t_{1/2}$  = 13 h, CL/F = 1.2 L/h). After 5 weeks, the decline in HbA<sub>1c</sub> was greater with liraglutide vs. placebo (-0.86 vs 0.04%,  $p=0.0007$ ), while mean body weight remained stable (-0.50 vs -0.54 kg,  $p=0.9703$ ).

**Conclusion:** Liraglutide was well-tolerated in a paediatric cohort with T2D, with safety, tolerability and PK parameters similar to those in adults.

**Table.** Summary of gastrointestinal AEs, hypoglycaemic events by liraglutide dose and by randomised group and calcitonin levels.

	Lira 0.3 mg	Lira 0.6 mg	Lira 0.9 mg	Lira 1.2 mg	Lira 1.8 mg	Total lira	Total placebo
	N (%) E	N (%) E	N (%) E	N (%) E	N (%) E	N (%) E	N (%) E
Subjects (n)	14	12	9	9	9	14 (100)	7 (100)
Gastrointestinal disorders*	5 (35.7) 9	3 (25.0) 4	1 (11.1) 1	1 (11.1) 1	2 (22.2) 2	8 (57.1) 17	2 (28.6) 7
Diarrhoea	3 (21.4) 3	3 (25.0) 3	0 (0.0) 0	0 (0.0) 0	1 (11.1) 1	6 (42.9) 7	1 (14.3) 1
Nausea	3 (21.4) 3	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	1 (11.1) 1	3 (21.4) 4	1 (14.3) 1
Vomiting	0 (0.0) 0	1 (8.3) 1	1 (11.1) 1	0 (0.0) 0	0 (0.0) 0	2 (14.3) 2	2 (28.6) 3
Hypoglycaemic events							
Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Symptomatic	1 (7.1) 1	1 (8.3) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	1 (7.1) 2	0 (0.0) 0
Asymptomatic	1 (7.1) 1	3 (25.0) 8	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	3 (21.4) 9	1 (14.3) 1
Calcitonin levels (pmol/L)**						Mean (SD)	Mean (SD)
Males:							
Baseline						0.60 (0.54)	0.33 (0.12)
Post-treatment						0.55 (0.50)	0.18 (0.11)
Females:							
Baseline						0.13 (0.10)	0.10 (0.0)
Post-treatment						0.17 (0.14)	0.10 (0.0)

n=number of subjects, %=proportion of subjects, E=number of events, lira=liraglutide; SD=standard deviation

\*Total numbers include 7 classes of events; 3 classes with the highest numbers of events are presented

\*\*Calcitonin levels were measured before treatment and after the end of treatment for total liraglutide and placebo arms

Normal calcitonin range (pmol/L): males (3–18 years) ≤3.51; females (3–18 years) ≤1.46

Safety parameters were not statistically analysed

Clinical Trial Registration Number: NCT00943501

Supported by: Novo Nordisk

2

### The once-weekly human GLP-1 analogue semaglutide provides significant reductions in HbA<sub>1c</sub> and body weight in patients with type 2 diabetes

M.A. Nauck<sup>1</sup>, J.R. Petrie<sup>2</sup>, G. Sesti<sup>3</sup>, E. Mannucci<sup>4</sup>, J.-P. Courrèges<sup>5</sup>, S. Atkin<sup>6</sup>, M. Düring<sup>7</sup>, C.B. Jensen<sup>7</sup>, S. Heller<sup>8</sup><sup>1</sup>Diabetes Centre, Bad Lauterberg im Harz, Germany, <sup>2</sup>University of Glasgow,UK, <sup>3</sup>University Magna Graecia, Catanzaro, Italy, <sup>4</sup>Careggi TeachingHospital, Florence, Italy, <sup>5</sup>General Hospital, Narbonne, France, <sup>6</sup>Hull YorkMedical School, Hull, UK, <sup>7</sup>Novo Nordisk, Søborg, Denmark, <sup>8</sup>University of

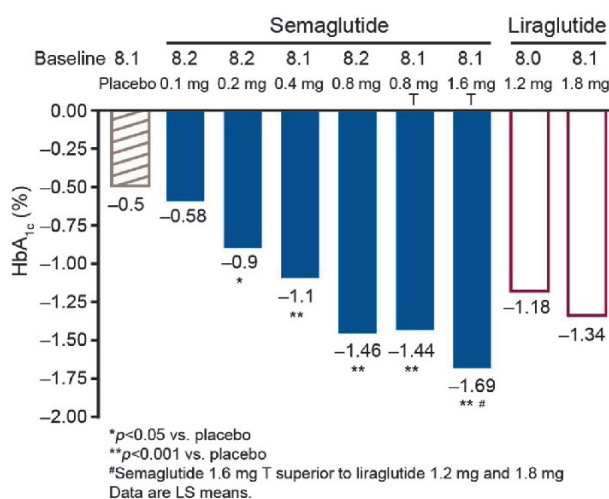
Sheffield, UK.

**Background and aims:** Semaglutide is a unique acylated human GLP-1 analogue with a half life of 160 h. The aim was to investigate HbA<sub>1c</sub> dose response of once weekly (OW) doses of semaglutide in subjects with T2D. Safety, tolerability and pharmacodynamics of semaglutide vs placebo and open label once daily (OD) liraglutide were also investigated.

**Materials and methods:** In a 12-week, randomised, double-blind, placebo-controlled trial, 411 subjects (n=43-50 per group) with T2D were exposed. Participants (male/female 65/35%; HbA<sub>1c</sub> (mean±SD) 8.1±0.8%; body weight 87.5±13.8 kg; diabetes duration 2.6±3.1 years; met only/diet & exercise alone 80/20%) received sc injection of one of five semaglutide doses (0.1-1.6 mg) OW, open label liraglutide (1.2 mg, 1.8 mg) OD or placebo OW. Two semaglutide doses were titrated (T) in weekly increments of 0.4 mg. Primary endpoint was change in HbA<sub>1c</sub> from baseline. Secondary efficacy endpoints included proportion of subjects reaching ADA HbA<sub>1c</sub> target (<7%) and change in body weight. Change and percentage to target were analysed by ANOVA and logistic regression, respectively. Comparisons between semaglutide and liraglutide were not multiplicity-corrected.

**Results:** Semaglutide ≥0.2 mg dose dependently reduced HbA<sub>1c</sub> from baseline (Fig), and increased the likelihood of achieving HbA<sub>1c</sub> <7% ( $p<0.05$  vs placebo for doses ≥0.2 mg). Treatment with semaglutide ≥0.8 mg numerically brought more patients to target than liraglutide 1.8 mg (0.8 mg T 69%, 0.8 mg 73%, 1.6 mg T 81% vs liraglutide 1.8 mg 57%). Body weight was dose dependently reduced from baseline by up to 4.8 kg vs placebo 1.2 kg ( $p<0.01$  for doses ≥0.8 mg). There were no reports of pancreatitis or treatment-related changes in blood calcitonin. Proportion of subjects with nausea and vomiting increased with dose, but events were generally mild or moderate and ameliorated by titration. Withdrawals due to gastrointestinal AEs were: placebo 0%; liraglutide 1.2 mg 2.2%, 1.8 mg 10%; semaglutide 0.1 mg 0%, 0.2 mg 4.7%, 0.4 mg 8.3%, 0.8 mg T 18.6%, 0.8 mg 14.3%, and 1.6 mg T 27.7%. Few subjects reported minor hypoglycaemia (semaglutide n = 5, liraglutide n = 3); no major hypoglycaemia. Injection site reactions were reported by 7 subjects: semaglutide n = 2; liraglutide n = 5. One subject (semaglutide 1.6 mg T) developed low titre non-neutralising anti-semaglutide antibodies (no crossreaction to native GLP-1).

**Conclusion:** Over 12 weeks, semaglutide dose dependently reduced HbA<sub>1c</sub> and body weight, with higher doses being more effective than liraglutide. No semaglutide safety concerns were identified. Future trials will attempt to further reduce side effects with effective dose escalation regimens.



Clinical Trial Registration Number: NCT00696657

Supported by: Novo Nordisk

## 3

### Efficacy and safety of once-daily lixisenatide in type 2 diabetes insufficiently controlled with basal insulin ± metformin: GetGoal-L study

R. Aronson<sup>1</sup>, M. Riddle<sup>2</sup>, P. Home<sup>3</sup>, M. Marre<sup>4</sup>, E. Niemoeller<sup>5</sup>, L. Ping<sup>6</sup>, J. Rosenstock<sup>7</sup>

<sup>1</sup>LMC Endocrinology Centres, Toronto, Canada, <sup>2</sup>Oregon Health and Science University, Portland, USA, <sup>3</sup>Newcastle University, Newcastle upon Tyne, UK, <sup>4</sup>Université Paris 7, INSERM U695, France, <sup>5</sup>Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, <sup>6</sup>Sanofi, Bridgewater, USA, <sup>7</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, USA.

**Background and aims:** This study used a randomized, placebo-controlled, double-blind, multicentre study design over 24 weeks to evaluate the benefit of adding once-daily lixisenatide to established and stable basal insulin therapy ± metformin, in people with Type 2 diabetes mellitus (T2DM) and inadequate blood glucose control.

**Materials and methods:** The modified intention-to-treat (mITT) population included 493 participants randomized 2:1 to injectable lixisenatide 20 µg or placebo each morning. Insulin dosage was kept constant throughout the study, within 20% of the daily dose at screening. If HbA<sub>1c</sub> was ≤7.5% at entry, insulin could be temporarily reduced by 20%. The primary endpoint was HbA<sub>1c</sub> reduction with lixisenatide versus placebo at endpoint (Week 24 or last observation carried forward).

**Results:** Baseline demographics: mean T2DM duration 12.5 years, BMI 32.1 kg/m<sup>2</sup>, HbA<sub>1c</sub> 8.4% and were balanced between both groups. Previous insulin therapy (mean 55 U/day) included glargine (50%), NPH (40%), detemir (9%), premix (2%). Compared with placebo, lixisenatide reduced HbA<sub>1c</sub> (-0.36%; p<0.001), 2-h postprandial glucose after standardized breakfast (-3.81 mmol/L; p<0.0001), and weight (-1.28 kg; p<0.0001) (Table). More lixisenatide versus placebo patients achieved HbA<sub>1c</sub> <7.0% (28 vs 12%; p<0.0001). However, insulin dose at endpoint decreased more with lixisenatide versus placebo (-5.6 vs -1.9 U/day; p=0.012). Incidences of all and serious adverse events were 73.5 and 3.7% with lixisenatide versus 68.3 and 4.2% with placebo. Discontinuation due to adverse events (mainly gastrointestinal) was 7.6% with lixisenatide and 4.8% with placebo. Comparable proportions of lixisenatide versus placebo participants had a documented hypoglycaemic event (27.7 vs 21.6%); four (1.2%) had severe hypoglycaemia with lixisenatide, none with placebo.

**Conclusion:** In patients with uncontrolled T2DM using basal insulin ± metformin, addition of once-daily lixisenatide therapy produced improved glycaemic control (despite ~10% reduction in insulin dose) and reduced body weight. Adverse events were mainly gastrointestinal and tended to subside over time.

Efficacy parameters in mITT population		Lixisenatide (n=327)	Placebo (n=166)
HbA <sub>1c</sub> (%)	Mean baseline ± SD	8.39 ± 0.86	8.38 ± 0.83
	LS mean ± SE change from baseline	-0.74 ± 0.09	-0.38 ± 0.11
	LS mean difference vs placebo	-0.36 (-0.55 to -0.17) p<0.001	
2-hour post-breakfast plasma glucose (mmol/L)*	Mean baseline ± SD	16.44 ± 4.29	15.85 ± 3.71
	LS mean ± SE change from baseline	-5.54 ± 0.47	-1.72 ± 0.54
	LS mean difference vs placebo	-3.81 (-4.70 to -2.93) p<0.0001	
Body weight (kg)	Mean baseline ± SD	87.39 ± 20.00	89.01 ± 21.00
	LS mean ± SE change from baseline	-1.80 ± 0.25	-0.52 ± 0.29
	LS mean difference vs placebo	-1.28 (-1.80 ± -0.75) p<0.0001	
Safety parameters in safety population, n (%)			
Symptomatic hypoglycaemia†		91 (27.7)	36 (21.6)
Nausea		86 (26.2)	14 (8.4)
Vomiting		27 (8.2)	1 (0.6)
Diarrhoea		24 (7.3)	9 (5.4)

\*After a standardized meal test; <sup>†</sup>Event with clinical symptoms with either plasma glucose <3.3 mmol/L or prompt recovery after carbohydrate administration if no plasma glucose measurement was available: mITT=modified intention to treat; SD=standard deviation; LS=least squares; SE=standard error

Clinical Trial Registration Number: NCT00715624

Supported by: Sanofi

## 4

### Exenatide once weekly resulted in sustained improvement in glycaemic control with weight loss through four years

D. Maggs, L. Porter, Y. Li, R. Pencek, J. Zhou, C. Schulteis, L. MacConell; Amylin Pharmaceuticals, Inc., San Diego, USA.

**Background and aims:** In the 30-wk, randomised, open-label DURATION-1 study, the once-weekly formulation of the GLP-1 receptor agonist exenatide (EQW) significantly reduced HbA<sub>1c</sub> compared with twice-daily exenatide (LS mean Δ HbA<sub>1c</sub>: -1.9% vs. -1.5%; p=0.002), with similar weight loss, in 295 intent-to-treat (ITT) patients with Type 2 diabetes mellitus treated with diet/exercise, metformin, sulphonylurea (SU), and/or thiazolidinedione. Following the controlled period of the study, long-term efficacy and safety were evaluated in an open-label extension period, during which all subjects received EQW, for a total of 4 years.

**Materials and methods:** Of the original 295 ITT patients participating in DURATION-1, 258 (87%) received EQW in the long-term extension period. Of those participating in the extension, 176 (68%) patients completed 4 y of EQW treatment (baseline [mean±SD]: HbA<sub>1c</sub> 8.2±0.9%; FPG 9.2±2.3 mmol/L; weight 100±18 kg; duration of diabetes 7±5 y). During the extension period, some patients increased or decreased oral antidiabetes medication doses.

**Results:** Long-term EQW treatment was associated with significant HbA<sub>1c</sub> reduction (LS mean [95%CI]: -1.7% [-1.9, -1.5]). At 4 y, mean±SE HbA<sub>1c</sub> was 6.9±0.1%, with 55% of patients achieving HbA<sub>1c</sub> <7.0% and 36% achieving HbA<sub>1c</sub> ≤6.5%. Clinically significant improvements in FPG (-2.1 mmol/L [-2.4, -1.7]) and weight (-2.5 kg [-3.8, -1.2]) were observed. A total of 34% and 22% patients achieved the goal of HbA<sub>1c</sub> <7% and ≤6.5%, respectively, accompanied by weight loss and with no reported hypoglycaemia events. Improvements were also observed from baseline to 4 y in cardiovascular risk markers: systolic blood pressure (LS mean [95%CI]: -1.6 mmHg [-4.0, 0.9]), total cholesterol (-0.28 mmol/L [-0.43, -0.13]), LDL cholesterol (-0.21 mmol/L [-0.33, -0.08]), and triglycerides (-13% [-19, -6]; geometric LS mean % change). Nausea, the most common adverse event (AE) with EQW during the initial controlled period, decreased in incidence with ongoing therapy, as did injection-site pruritus, the most frequent injection-site related adverse event observed during the controlled period. The annual event rate for nausea with EQW was 15/100 y patient exposure over the 4-y study duration. Injection-site pruritus occurred at an annual event rate of 6/100 y patient exposure over the 4-y study duration. Six (2%) EQW patients withdrew due to gastrointestinal AEs and 1 (<1%) EQW patient withdrew due to an injection-site related adverse event over 4 y. No major hypoglycaemia was observed. Among EQW patients using SU at baseline, minor hypoglycaemia was observed at an annual event rate of 137/100 y patient exposure over 4 y. In EQW patients not using concomitant SU at baseline, minor hypoglycaemia was observed at rate of 26/100 y patient exposure over 4 y. Annual event rates for cardiac and renal/urinary disorders were 5/100 y patient exposure and 6/100 y patient exposure, respectively, for EQW over 4 y.

**Conclusion:** Overall, longterm EQW treatment was associated with significant, sustained improvement in glycaemic control and improvements in cardiometabolic measures, with no unexpected safety findings.

Clinical Trial Registration Number: NCT00308139

## 5

### Therapy escalation options for patients failing therapy with exenatide BID + metformin or glimepiride + metformin: results from the EUREXA clinical study

B. Gallwitz<sup>1</sup>, J. Guzman<sup>2</sup>, F. Dotta<sup>3</sup>, B. Guerci<sup>4</sup>, R. Simo<sup>5</sup>, B. Basson<sup>6</sup>, A. Festa<sup>7</sup>, J. Kiljanski<sup>8</sup>, H. Sapin<sup>9</sup>, S. Chen<sup>10</sup>, M. Trautmann<sup>11</sup>, G. Scherthaner<sup>12</sup>; <sup>1</sup>Eberhard-Karls University, Tübingen, Germany, <sup>2</sup>Celaya Center for Specialist Medicine, Guanajuato, Mexico, <sup>3</sup>Policlinico Le Scotte, Siena, Italy, <sup>4</sup>Brabo Hospital & CIC, Nancy, France, <sup>5</sup>Vall d'Hebron Research Institute, Barcelona, Spain, <sup>6</sup>Lilly France, St Cyr au Mont D'Or, France, <sup>7</sup>Lilly Austria, Vienna, Austria, <sup>8</sup>Lilly Polska, Warsaw, Poland, <sup>9</sup>Lilly France, Paris, France, <sup>10</sup>Amylin Pharmaceuticals, Inc., San Diego, USA, <sup>11</sup>Lilly Deutschland, Hamburg, Germany, <sup>12</sup>Rudolfstiftung Hospital, Vienna, Austria.

**Background and aims:** Type 2 diabetes continues to progress despite therapy, and treatment options when one therapy fails remain controversial. The study aim was to examine escalation with exenatide twice daily (EXE) or glimepiride once daily (GLI) in patients with metformin (MET) failure, and subsequent escalation when these agents failed to maintain HbA<sub>1c</sub> control.



**Materials and methods:** In a long-term (up to 54 mo) randomised open-label study, the primary comparison was EXE (N=490) vs GLI (N=487) as add-on for patients (mean age 56 y, BMI 32.5 kg/m<sup>2</sup>, diabetes duration 6 y, HbA1c 7.4%) with inadequate glycaemic control with MET. Primary endpoint was time to inadequate HbA1c control (HbA1c >9% at any visit or >7% at 2 successive visits, excluding first follow-up visit). Patients with inadequate control were recruited to an extension phase: patients on EXE were re-randomized to add-on GLI (EXE+GLI) or thiazolidinedione (EXE+TZD); patients on GLI received add-on EXE (GLI+EXE).

**Results:** Estimated hazard ratio for time to inadequate control (EXE/GLI Cox regression adjusted for baseline HbA1c) was 0.748 (95% CI 0.623, 0.899,  $P=0.002$ ), indicating superiority of EXE. Rate of documented hypoglycaemia (hypo) was lower for EXE vs GLI (0.76 vs 2.43 episodes/y). Data for patients who received extension add-on therapy after inadequate control on EXE or GLI are shown in Table. HbA1c decreased for EXE+TZD and increased for EXE+GLI, with significant difference from 18 mo to at least 30 mo. HbA1c decreased for GLI+EXE. BMI was unchanged for EXE+GLI and increased for EXE+TZD (difference significant from 24 mo onwards) and decreased for GLI+EXE. Rate of documented hypo was lowest in the EXE+TZD group ( $P<0.001$  vs EXE+GLI).

**Conclusion:** In EUREXA, for patients using MET, add-on therapy with EXE was superior to GLI in maintaining long-term HbA1c control. In patients who developed inadequate HbA1c control with EXE+MET, additional TZD was effective in reducing HbA1c, with low hypo risk but increased body weight. Addition of GLI to EXE+MET did not improve HbA1c (possibly due to beta-cell failure associated with disease progression) and increased hypo risk. Addition of EXE was effective in reducing HbA1c and weight in patients failing therapy with GLI+MET although hypo was observed. These results support use of TZD but not GLI as the third therapy for HbA1c control in patients losing glycaemic control after long-term therapy with EXE+MET.

Initial randomised therapy	EXE		GLI
Extension therapy	EXE+GLI (N=74)	EXE+TZD (N=76)	GLI+EXE (N=166)
Exenatide exposure during extension (wk)	112±42	109±51	99±52
Baseline HbA1c at extension start (%)	7.59±0.55	7.74±0.68	7.67±0.77
HbA1c change to endpoint (%)	0.16±0.99	-0.22±1.03	-0.19±1.01
HbA1c ≤6.5%, n (%)	6 (8.2)	7 (9.3)	28 (17.7)
HbA1c ≤7.0%, n (%)	19 (26.0)	23 (30.7)	54 (34.2)
Baseline BMI at extension start (kg/m <sup>2</sup> )	32.6±4.1	32.8±4.3	33.2±4.2
BMI change to endpoint (kg/m <sup>2</sup> ) *	0.1±1.5	0.7±1.6	-1.2±1.6
Documented hypo (episodes/y) †	1.36±3.20	0.13±0.58	2.51±8.35

\*Significant difference between re-randomized EXE+GLI and EXE+TZD groups by ANCOVA.

†Significant difference between re-randomized EXE+GLI and EXE+TZD by negative binomial model.

Clinical Trial Registration Number: NCT00359762;

EudraCT Registration Number: 2005-005448-21

## 6

### Long-term safety and efficacy of linagliptin as add-on therapy to basal insulin in patients with type 2 diabetes: a 52-week randomised, placebo-controlled trial

H. Yki-Järvinen<sup>1</sup>, J. Rosenstock<sup>2</sup>, S. Durán-García<sup>3</sup>, S. Pinnett<sup>4</sup>, S. Bhattacharya<sup>4</sup>, S. Thiemann<sup>5</sup>, S. Patel<sup>6</sup>, H.-J. Woerle<sup>5</sup>;

<sup>1</sup>Dept of Medicine, Helsinki, Finland, <sup>2</sup>Dallas Diabetes and Endocrine Center, Dallas, USA, <sup>3</sup>Valme University Hospital Medical School, Seville, Spain, <sup>4</sup>Boehringer Ingelheim, Biberach, Germany, <sup>5</sup>Boehringer Ingelheim, Ingelheim, Germany, <sup>6</sup>Boehringer Ingelheim, Bracknell, UK.

**Background and aims:** Titrated basal insulin added to metformin +/- pioglitazone improves glucose control close to glycaemic targets in patients with type 2 diabetes. Adding a DPP-4 inhibitor instead of a sulphonylurea to further improve glucose control is a sensible therapy option to avoid hypoglycaemia and weight gain. This multicentre, randomised, placebo-controlled, Phase 3 study evaluated the long-term safety and efficacy of the DPP-4 inhibitor linagliptin as add-on therapy to basal insulin alone or in combination with metformin and/or pioglitazone. The primary efficacy endpoint - mean change in HbA1c from baseline to Week 24 - was reported recently. Here we

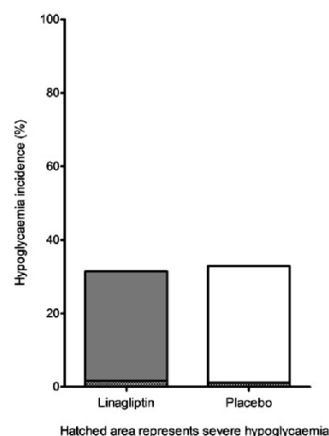
report sustained glucose control and long-term safety data with an emphasis on hypoglycaemia.

**Materials and methods:** A total of 1261 patients inadequately controlled on insulin glargine, insulin detemir, or NPH insulin were randomised to receive linagliptin 5 mg qd or placebo (1:1) for at least 52 weeks. The background dose of basal insulin was kept stable up to 24 weeks but could be freely adjusted after this period.

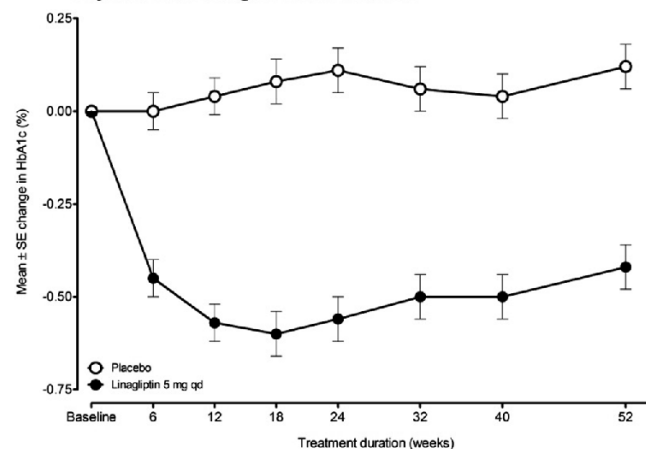
**Results:** Mean ± SD baseline characteristics were similar in the linagliptin vs placebo groups: age, 59.7 ± 9.9 vs 60.4 ± 10.0 yrs; BMI, 30.8 ± 5.4 vs 31.2 ± 5.0 kg/m<sup>2</sup>; HbA1c, 8.3% ± 0.9% both; basal insulin dose, 42 ± 32 vs 40 ± 27 IU/day. Mean exposure to study medication exceeded 52 weeks and was comparable in both groups: linagliptin 435 days; placebo 422 days. Overall safety and tolerability for linagliptin was similar to placebo. The proportion of patients with ≥1 adverse event (AE) was slightly lower with linagliptin (78.4%) compared with placebo (81.4%) and most AEs were of mild or moderate intensity. The incidence of hypoglycaemia (Figure A) was similar in both groups (linagliptin 31.4%; placebo 32.9%) despite better glycaemic control with linagliptin, and the number of severe hypoglycaemic events was low (linagliptin 1.7%; placebo 1.1%). Mean ± SD change in body weight was minimal and comparable between the treatment groups (linagliptin -0.30 ± 3.7 kg; placebo -0.04 ± 3.1 kg). The placebo-adjusted mean ± SE change in HbA1c from baseline at Week 52 for linagliptin (Figure B) was -0.53% ± 0.05 (full analysis set;  $P<0.0001$ ). This was accompanied by a mean ± SE change in basal insulin dose up to Week 52 of +2.6 ± 0.8 IU/day for linagliptin vs +4.2 ± 0.8 IU/day for placebo ( $P<0.003$ ).

**Conclusion:** The addition of linagliptin to patients inadequately controlled on non-optimized basal insulin therapy significantly improves long-term glycaemic control over 52 weeks with no additional risk of hypoglycaemia and no weight gain.

**Figure (A)**  
Investigator-defined hypoglycaemia



**Figure (B)**  
Adjusted mean change in HbA1c over time



Clinical Trial Registration Number: NCT00954447

Supported by: Boehringer Ingelheim

## OP 02 Impact of bariatric surgery

7

### Reversal of type 2 diabetes following gastric bypass surgery is determined by the degree of achieved weight loss and is not limited by disease duration

S. Steven<sup>1</sup>, J.-A. Coates<sup>2</sup>, P. Ogilvie<sup>2</sup>, P.E. Carey<sup>2</sup>, P.K. Small<sup>3</sup>, R. Taylor<sup>1</sup>;<sup>1</sup>Magnetic Resonance Centre, Newcastle University, Newcastle upon Tyne,<sup>2</sup>Metabolic Medicine Unit, Sunderland Royal Hospital, <sup>3</sup>Department of Surgery, Sunderland Royal Hospital, UK.

**Background and aims:** Bariatric surgery is currently the most effective tool to reverse type 2 diabetes. Roux-en-Y gastric bypass is now the most common procedure worldwide. It is widely believed that long duration type 2 diabetes results in an irreversible decline in beta cell function and previous studies have suggested that long duration diabetes is less likely to revert to normal after bariatric surgery. However, these data may have been confounded by the inclusion of individuals with alternative diagnoses to type 2 diabetes, such as MODY or slow onset type 1 diabetes. We have previously demonstrated the pathophysiological mechanism of diabetes reversal following acute food restriction. The aim of this study was to define the influence of diabetes duration and achieved weight loss on reversal of type 2 diabetes.

**Materials and methods:** Data were collated on consecutive referrals to the diabetes team at a specialist bariatric surgery centre (2009–2011). 92 individuals with type 2 diabetes underwent Roux-en-Y gastric bypass surgery. The diagnosis of type 2 diabetes was verified in all individuals. Reversal of diabetes was defined as achieving a post-operative HbA<sub>1c</sub> of <6.1% or 43 mmol/mol. Diabetes duration was defined as short (<4 years) or long (>8 years). Follow-up information on use of anti-diabetic medications was requested from general practitioners. Results are expressed as mean ± SD or median and range when not normally distributed. Two sample t-test and Spearman rank sum were tested using Minitab 15 Statistical Software.

**Results:** 34 males and 58 females were identified; age 49.1 ± 9.2 years, pre-operative BMI 47.3 kg/m<sup>2</sup> (34.8–78.2) and HbA<sub>1c</sub> 7.1% (5.3–14.0). Median diabetes duration at the time of surgery was 5 years (1 month–22 years). Prior to surgery, diabetes treatments were: metformin (76); sulphonylurea (32); insulin (18); thiazolidinedione (16); glucagon-like peptide-1 agonist (15); dipeptidyl peptidase-4 inhibitor (6). Diabetes reversal occurred in 79% of those with short duration disease and 38% of those with long duration disease. Of these, 85% and 86% respectively (with available follow-up data) were confirmed to be off anti-diabetic medications. Post-operative decrease in BMI was the major predictor of diabetes reversal: <10 kg/m<sup>2</sup>: 53%; 10–15 kg/m<sup>2</sup>: 74%; and >15 kg/m<sup>2</sup>: 82% reversal. The achieved post-operative HbA<sub>1c</sub> correlated with the percentage body weight loss (Rs = -0.293; p-value = 0.008) and also the absolute weight loss (Rs = -0.307; p-value = 0.006). In those achieving weight loss of more than 20% of initial body weight, the reversal rates in the short and long diabetes duration groups were 87% and 56% compared to 70% and 25% in those achieving less than 20% weight loss. In the long duration group, the mean weight loss in those who reversed (median duration 11 years, range 10–18) was 34.5 ± 13.4 kg compared to 21.5 ± 9.4 kg in those who did not reverse (median duration 12 years, range 9–19) (T-value = 2.4; p-value = 0.035).

**Conclusion:** Duration of type 2 diabetes is no bar to reversal of type 2 diabetes, and long duration disease can be reversed by Roux-en-Y gastric bypass surgery. The degree of achieved weight loss is the main determinant of outcome. Individuals with long duration disease require greater weight loss in order to reverse type 2 diabetes.

8

### Insulin resistance in femoral bone marrow fat of diabetic morbidly obese patients is alleviated after bariatric surgery

T.T. Pham<sup>1</sup>, R. Kiviranta<sup>2</sup>, J.C. Hannukainen<sup>1</sup>, H. Immonen<sup>1</sup>, P. Salminen<sup>3</sup>, P. Nuutila<sup>1,4</sup>;<sup>1</sup>Department of Medicine, Turku PET center, <sup>2</sup>Department of Medicine, Department of Medical Biochemistry and Genetics, <sup>3</sup>Department of Surgery, Turku University Hospital, <sup>4</sup>Department of Medicine, Turku University Hospital, Turku, Finland.

**Backgrounds and aims:** In adults, majority of bone marrow space of long bones is filled with fat tissue. Obesity is linked to increased bone marrow adiposity and insulin resistance. We have recently found that glucose uptake (GU) in femoral bone marrow fat is responsive to insulin. Our aim in this

study was to measure glucose uptake in femoral and vertebral bone marrow during fasting and hyperinsulinemic euglycemic clamp in morbidly obese and type 2 diabetes patients. Further, we tested whether bariatric surgery would alter bone marrow glucose uptake.

**Materials and methods:** Twenty-three morbidly obese patients (BMI 42.6 ± 3.5 kg/m<sup>2</sup>), 9 non-diabetics and 14 with prediabetes or type 2 diabetes (DM), were studied before and 6 months after bariatric surgery. GU in muscle, subcutaneous and visceral fat, and vertebral and femoral bone marrow was measured with <sup>18</sup>F-FDG and Positron Emission Tomography (PET) at fast and during hyperinsulinemic euglycemic clamp (1 mU/kg/min) before and 6 months after the bariatric surgery.

**Results:** Obese patients were similarly insulin resistant in skeletal muscle and whole body before the operation. At fasting state, GU in femoral bone (8.7 ± 3.2 μmol/l/min) was similar to muscle and visceral GU and 2.5 times higher than subcutaneous fat. Insulin increased femoral bone marrow adipose GU by 90% in obese without DM (to 16 ± 1 μmol/kg/min, p = 0.02), but not in those with DM. Vertebral bone marrow GU was high already at baseline (26.4 ± 4.5 μmol/l/min) and not altered by insulin. After the operation, patients lost weight by 27.5 ± 5.2 kg (p < 0.001) and improved whole body insulin sensitivity (DM+ by 119 %, DM- by 81 %, p < 0.001, NS between subgroups). Femoral bone GU responded to insulin postoperatively by 134% (p = 0.03) and 187% (p = 0.001) in non-DM and DM patients.

**Conclusion:** This study shows that fatty femoral bone marrow is one site of insulin resistance in patients with type 2 diabetes and obesity, while vertebral bone marrow consisting mostly of hematopoietic cells is not. Femoral bone adipose tissue insulin resistance is reversed 6 months after bariatric surgery. Further studies are needed to assess the effects of insulin sensitizers and high daily insulin dosage on bone adipose metabolism.

Clinical Trial Registration Number: NCT00793143

Supported by: EU FB6 (18734, Hepadip), Academy of Finland, Sigrid Juselius, VKTK

9

### Glycaemic control after endoscopically placed duodenal-jejunal bypass liner in patients with type 2 diabetes and body mass index between 25 and 35 kg/m<sup>2</sup>

D.J. Pournaras<sup>1</sup>, R.V. Cohen<sup>2</sup>, C.W. le Roux<sup>1</sup>, D. Papamargaritis<sup>1</sup>, J. Salles<sup>2</sup>, T. Petry<sup>2</sup>, J. Correa<sup>2</sup>, M.G. Neto<sup>2</sup>, B. Martins<sup>2</sup>, P. Sakai<sup>2</sup>, C.A. Schiavon<sup>2</sup>, C. Sorli<sup>3</sup>;<sup>1</sup>Imperial Weight Center, Imperial College London, UK, <sup>2</sup>Center of Excellence for the Surgical Treatment of Morbid Obesity, Hospital Oswaldo Cruz, São Paulo, Brazil, <sup>3</sup>Billings Clinic, Billings, USA.

**Background and aims:** The duodenal-jejunal bypass liner (DJBL, EndoBarrier™, GI Dynamics Inc., Lexington, MA) is a removable device which is endoscopically placed. It was designed with the objective to mimic the effects of the bypass of the proximal gut in the Roux-en-Y gastric bypass and Duodenal Jejunal Bypass without the risks associated with surgery. The aim of this study was to investigate the effect of this novel device on glycemic control outcomes and explore its mechanism of action.

**Materials and methods:** Sixteen patients with type 2 diabetes and BMI < 35 kg/m<sup>2</sup> were included in this study. Time points were before and one, 12 and 52 weeks after implantation of the device. A standard mixed meal test was given and blood samples were collected prior to and 30, 60, 90 and 120 minutes after the meal. Glucose, insulin and C-peptide were measured. Insulin sensitivity was calculated using the Matsuda Index and the Homeostatic Model of Assessment Insulin Resistance (HOMA-IR). Insulin secretion was calculated using fasting and total insulin secretion rate after the meal by deconvolution of C-peptide concentrations. First phase insulin release was estimated by the acute insulinogenic index.

**Results:** One year post implantation, BMI, HbA<sub>1c</sub>, fasting and 120 min glucose levels were reduced (all p < 0.001). Insulin sensitivity as measured by the Matsuda Index and HOMA-IR improved significantly as early as 1 week post-implantation (p < 0.01). Fasting (p = 0.053) and area under the curve (p = 0.13) insulin, fasting (p = 0.28) and area under the curve C-peptide (p = 0.31), fasting (p = 0.27) and total (p = 0.81) insulin secretion rate, and insulinogenic index (p = 0.43) remained unchanged over the one year period of the implantation of the device.

**Conclusion:** Implantation of DJBL leads to improved glycemic control in overweight and obese type 2 diabetic patients due to improved insulin sensitivity observed in the early post implantation period and is sustained over one year. DJBL has no effect on insulin production.

## 10

**Glucagon-like-peptide-1 (GLP-1) is important for the improved beta cell function in type 2 diabetic subjects after Roux-en-Y gastric bypass (RYGB)**N.B. Jorgensen<sup>1,2</sup>, C. Dirksen<sup>1</sup>, S.H. Jacobsen<sup>1</sup>, K.N. Bojsen-Møller<sup>1</sup>, D.L. Hansen<sup>1</sup>, D. Worm<sup>1</sup>, S. Madsbad<sup>1</sup>, J.J. Holst<sup>2,3</sup><sup>1</sup>Dep. of Endocrinology, Hvidovre Hospital, <sup>2</sup>Dept of Biomedical Science, University of Copenhagen, <sup>3</sup>Novo Nordisk Foundation Centre for Basic Metabolic Research, University of Copenhagen, Denmark.

**Background and aims:**  $\beta$  cell function is known to improve in type 2 diabetic (T2D) subjects after RYGB, but whether this is explained by an increased GLP-1 secretion is not known. We used the GLP-1 receptor specific blocker, exendin (9-39) (EX9), to evaluate the role of GLP-1 on post RYGB  $\beta$  cell function.

**Materials and methods:** A liquid meal test was performed on two separate days before and 1 wk and 3 mo after RYGB in 7 subjects with T2D scheduled for RYGB surgery (Age:  $48.6 \pm 4.3$ ; BMI:  $36.9 \pm 0.77$  kg/m<sup>2</sup>). Fasting blood samples were drawn followed by a bolus-infusion of EX9 (900 pmol/kg/min) or isotonic saline (NaCl). The order of EX9 and NaCl infusion was randomized. After 30 min of infusion, the meal was ingested over 30 min, and blood was sampled frequently from 30 min before to 4 h after meal start. Prehepatic insulin secretion rates (ISR) were calculated by deconvolution of peripheral C-peptide concentrations and the application of population based parameters for C-peptide kinetics using the ISEC software.  $\beta$ -cell glucose sensitivity ( $\beta$ -GS) was calculated by cross correlating ISR and the corresponding glucose concentrations, and this was used for characterizing  $\beta$ -cell function.

**Results:** After RYGB fasting P-glucose (pre:  $8.5 \pm 0.94$  mM, 1 wk:  $7.0 \pm 0.57$  mM ( $p=0.08$ ), 3 mo:  $6.1 \pm 0.3$  mM ( $p<0.05$ ) and S-insulin ( $127 \pm 17$  pM,  $66 \pm 4$  pM ( $p<0.05$ ),  $59 \pm 7$  pM ( $p<0.05$ )) decreased. HOMA-IR was halved after RYGB ( $6.5 \pm 0.6$ ,  $3.0 \pm 0.4$  ( $p<0.05$ ),  $2.3 \pm 0.3$  ( $p<0.05$ )) and did not differ between the day of EX9 infusion and the day of NaCl infusion neither pre nor 1 wk nor 3 mo post RYGB. Before RYGB the area-under the glucose curve (AUC) was  $2.3 \pm 0.2$  M $\cdot$ min and this had decreased to  $1.9 \pm 0.1$  M $\cdot$ min ( $p=0.08$ ) 1 wk and to  $1.7 \pm 0.1$  M $\cdot$ min ( $p<0.05$ ) 3 mo after the operation. EX9 caused AUC glucose to increase before and after RYGB ( $2.7 \pm 0.3$  M $\cdot$ min ( $p<0.05$ ),  $2.4 \pm 0.2$  M $\cdot$ min ( $p<0.05$ ),  $2.1 \pm 0.1$  M $\cdot$ min ( $p<0.05$ )).  $\beta$ -GS increased after RYGB on the days of NaCl infusion ( $1.1 \pm 0.3$  pmol/kg/mM,  $2.3 \pm 0.3$  pmol/kg/mM ( $p<0.01$ ),  $2.3 \pm 0.5$  pmol/kg/mM ( $p<0.05$ )). Compared to the corresponding day of NaCl infusion, EX9 infusion caused  $\beta$ -GS to decrease only after RYGB ( $0.9 \pm 0.2$  pmol/kg/mM ( $p=0.2$ ),  $1.2 \pm 0.3$  pmol/kg/mM ( $p<0.01$ ),  $1.1 \pm 0.3$  pmol/kg/mM ( $p<0.05$ )), to levels not different from the preoperative  $\beta$ -GS.

**Conclusion:** After RYGB glucose tolerance and beta-cell function is improved in type 2 diabetic subjects. Infusion of a GLP-1 receptor specific blocker causes beta-cell function to decrease to preoperative levels, and glucose tolerance to impair during a meal test. This suggests an important role for GLP-1 in the improved glucose metabolism of T2D patients after RYGB

Clinical Trial Registration Number: NCT00810823

Supported by: Desiree &amp; Niels Ydes Foundation

## 11

**Oral route versus gastrostomy route: effects on glucose metabolism and incretin response to a mixed meal in gastric-bypassed humans**A. Lindqvist<sup>1</sup>, M. Ekelund<sup>2</sup>, J. Hedenbro<sup>2</sup>, L. Groop<sup>1</sup>, N. Wierup<sup>1</sup><sup>1</sup>Clinical Sciences, Malmö, <sup>2</sup>Dept. of Surgery, Lund, Sweden.

**Background and aims:** Gastric bypass surgery (GBP) has beneficial effects on Type 2 diabetes (T2D), often resulting in a rapid normalization of blood glucose. The mechanisms behind the resolution of T2D after GBP remain unclear. Two hypotheses predominate; the foregut and the hindgut theories. The foregut theory states that the resolution of T2D is dependent on anti-incretins produced in the stomach and duodenum in response to nutrients. GBP prevents nutrient-stimulated anti-incretin stimulation, and incretins are allowed to work unopposed. The hindgut theory states that the rapid delivery of undigested nutrients to more distal parts of the intestine leads to up-regulation of the production of L-cell derived hormones, such as GLP-1. In this study, we aimed at exploring the role of the foregut in glucose tolerance and incretin response to a mixed meal in gastric-bypassed humans. We utilized a model where a gastrostomy tube (GT) was placed in the stomach. Infusing a mixed meal via the GT allowed us to study presurgical exposure to nutrients under conditions of postsurgical caloric restriction (weight loss).

**Materials and methods:** Four female patients having previously undergone GBP with an accompanying gastrostomy tube were given a mixed meal test orally, and thereafter the same amount of the test meal was infused via the GT. Blood was collected at regular intervals before and after the mixed meals. Glucose, insulin, glucagon, glucose-dependent insulintropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) were analyzed in serum or plasma.

**Results:** The oral administration route resulted in a robustly larger insulin response, compared to the GT route, at 5, 10, 15 and 30 minutes after the mixed meal (57%, 210%, 368%, 359% and 193%, respectively). Serum glucose was elevated at 5, 10, 15 and 30 minutes after oral ingestion of the mixed meal (10%, 10%, 23% and 14%, respectively, compared to GT infusion). Plasma glucagon was higher at 30 minutes after oral ingestion of the mixed meal (17% compared to GT infusion of the mixed meal). Insulin AUC:GLP-1 AUC-ratio was 2-fold higher when mixed meal was administered orally compared to mixed meal infused via the GT. Administering the mixed meal orally resulted in a 2-fold increase in Insulin AUC:GIP AUC compared to mixed meal infused via the GT (\*,  $p<0.05$ ).

**Conclusion:** In GBP patients, oral administration of a mixed meal triggered a higher response in insulin and GIP than did infusion via the GT. Whether this is due to the bypassing of the cephalic phase when administering the mixed meal via the GT route or the possibility that the secretory pattern of candidate anti-incretins in the proximal part of the intestine and the stomach may have been altered as a result of the gastric bypass surgery remain to be elucidated. In summary, the oral administration route evokes a more rapid and robustly larger insulin and incretin response than does the GT infusion. Thus, a rapid delivery to the hindgut may be of importance when considering the underlying mechanisms of the beneficial aspects of GBP on glucose metabolism.

Supported by: Swedish Diabetes Foundation, VR, ALF, The Pahlsson Foundation

## 12

**Does diabetes status affect hypertension resolution after bariatric surgery?**L. Flores<sup>1,2</sup>, J. Vidal<sup>1,2</sup>, S. Canivell<sup>3</sup>, L. Rodriguez<sup>1</sup>, E. Esmatjes<sup>4,3</sup><sup>1</sup>Obesity Unit, Hospital Clinic, <sup>2</sup>CIBERDEM <sup>3</sup>IDIBAPS, Hospital Clinic, Barcelona, <sup>4</sup>Diabetes Unit, Hospital Clinic, Barcelona, Spain.

Bariatric surgery is the most effective treatment for significant and sustained weight loss. On the other hand, weight loss is the main non-pharmacological therapy to control blood pressure in hypertensive-obese subjects. However, it has been demonstrated that less weight is lost in diabetic subjects after bariatric surgery (BS). The aims of this study were to evaluate the predictive factors associated with the risk of persistent hypertension and determine whether diabetes status affects hypertension resolution in a cohort of hypertensive-obese subjects after BS.

**Methods:** Weight, height, waist circumference and blood pressure were determined with standardized procedures. The body mass index (BMI), excess body weight (EBW) and EBW loss were calculated.

**Results:** We evaluated 530 patients undergoing BS between 2007-2009: 264 (50%) were hypertensive-obese (defined as having permanent antihypertensive treatment or office blood pressure  $\geq 140/90$  mmHg prior to bariatric surgery), 88 (33%) of whom had diabetes. Before BS older age, male gender and greater waist circumference differentiated hypertensive-obese from normotensive patients. Among all the hypertensive-obese subjects, the prevalence of hypertension significantly fell to 33% ( $p<0.05$ ) at 12 months after BS. Logistic regression models were constructed by gender to assess the variables associated with the risk of persistent hypertension after BS, and showed that men  $\geq 40$  years [OR 11.7 (95% CI 2.50-54.46,  $p=0.002$ )] and the number of antihypertensive drugs used [OR 3.29 (95% CI 1.59-6.82,  $p=0.001$ )] were independently associated with the risk of persistent hypertension. In women, time since hypertension diagnosis  $>10$  years [OR 3.96 (95% CI 1.47-10.67,  $p=0.007$ )] and the number of antihypertensive drugs used [OR 1.86 (95% CI 1.06-3.29,  $p=0.03$ )] were associated with the risk of persistent hypertension. Pre-surgical BMI, waist circumference, EBW, EBW loss and the type of surgical technique (gastric bypass or sleeve gastrectomy) were not significant predictors of persistent hypertension. The results were similar after excluding patients with diabetes, and EBW loss did not affect hypertension resolution in non diabetic patients.

**Conclusion:** BS is associated with a high rate of hypertension resolution, with older age, longer hypertension duration and severity of hypertension being predictors of hypertension persistence after BS. Diabetes status did not affect hypertension resolution.



## OP 03 Hyperglycaemia and the brain

### 13

#### Mannose-binding lectin level and cerebral white matter lesions in type 2 diabetes

P. Hoyem<sup>1</sup>, E. Laugesen<sup>1</sup>, S. Thrysoe<sup>2</sup>, U. Kampmann<sup>1</sup>, B. Stausbol-Gron<sup>2</sup>, M. Bjerre<sup>1</sup>, J.S. Christiansen<sup>1</sup>, W.Y. Kim<sup>2</sup>, T.K. Hansen<sup>1</sup>;

<sup>1</sup>Department of Endocrinology and Internal Medicine MEA, Denmark,

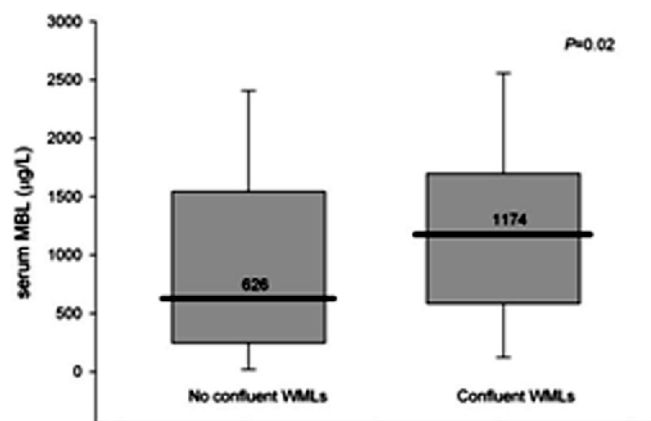
<sup>2</sup>MR Research Centre, Aarhus University Hospital, Aarhus, Denmark.

**Background and aims:** Mannose-binding lectin (MBL) serum levels are mainly genetically determined and in diabetes high levels are known to be associated with increased risk of a number of diabetic complications. Cerebral white matter lesions (WMLs), especially confluent WMLs, are associated with risk of cerebral infarction, a well-known diabetic complication. We examined the impact of MBL levels on WMLs assessed by magnetic resonance imaging (MRI) to further evaluate MBL as a prognostic and pathogenetic factor in diabetic complications.

**Materials and methods:** In a cross-sectional setting we included 100 patients with type 2 diabetes and time since diagnosis < 5 years (median age 59 years; males 52%), and 100 non-diabetic sex- and age-matched controls. We measured serum MBL and performed brain MRI. MRIs were subsequently qualitatively graded regarding WMLs from 0 to 2 on the Breteler-scale (0-4 punctate WMLs=0, >4 punctate WML but no confluent WMLs=1, presence of confluent WMLs regardless of number of punctate WMLs=2) by a trained radiologist blinded to participants status.

**Results:** Median MBL was 643 µg/L (inter-quartile range (IQR) 246-1419 µg/L) in patients and 798 µg/L (IQR 262-1667 µg/L) in controls, no difference between groups ( $P=0.52$ ). MBL correlated significantly to age (Spearman correlation,  $\rho=0.17$ ,  $P=0.02$ ), and was higher among men ( $P=0.007$ ), but was not related to duration of diabetes ( $P=0.16$ ). Distribution of Breteler score (0/1/2) was 59%/30%/11% among patients vs. 54%/32%/14% among controls ( $P=0.73$ ), and there was no difference in distribution between sexes. For all participants with MBL below median the distribution of Breteler score was 62%/32%/6% vs. 51%/31%/18% for all participants with MBL above median, the distributions differed significantly (Chi-Square  $p=0.03$ ), but the distribution did not differ significantly within groups (Chi-Square patients  $P=0.26$  and controls  $P=0.11$ ). Participants with confluent WMLs (Breteler score 2) had significantly higher median level of MBL 1174 µg/L (IQR 584-1698 µg/L) than participants without confluent WMLs (Breteler score 0+1) 626 µg/L (IQR 246-1545 µg/L) ( $P=0.02$ ), which was not the case within groups (patients  $P=0.19$ , controls  $P=0.07$ ).

**Conclusion:** In summary high serum MBL are significantly associated with the presence of WMLs in a mixed cohort of newly-diagnosed type 2 diabetic patients and non-diabetic control subjects. This association did not apply for the patient group, which could be expected as high MBL levels are known to be significantly associated with the risk of other diabetic complications. The missing association might be due to lack of power or to the short duration of diabetes.



Clinical Trial Registration Number: 20080059

### 14

#### Acute hyperglycaemia impairs working memory in women without diabetes but not in women with type 2 diabetes

A. Backeström<sup>1</sup>, T. Olsson<sup>2</sup>, S. Eriksson<sup>3</sup>, L. Nyberg<sup>4</sup>, L.-G. Nilsson<sup>5</sup>, O. Rolandsson<sup>1</sup>;

<sup>1</sup>Public Health and Clinical Medicine, Family Medicine, Umeå, <sup>2</sup>Public Health and Clinical Medicine, Medicine, Umeå, <sup>3</sup>Community Medicine and Rehabilitation, Geriatrics, Umeå, <sup>4</sup>Integrative Medical Biology, Radiation Sciences, Umeå, <sup>5</sup>Psychology, Stockholm, Sweden.

**Background and aims:** Metabolic dysfunction including type 2-diabetes is associated with increased risk for cognitive decline. Notably, it is debated whether acute hyperglycemia affects different brain regions and thus different memory functions. Thus, our aim was to study the effect of acute hyperglycemia on working, semantic and episodic memory in patients with type 2-diabetes (T2D) and non-diabetic participants (controls).

**Materials and methods:** Thirty-six (M/F, 18/18, mean±SD 66±2.3 yrs) patients with T2D and 34 (M/F, 17/17, mean age 66±0.6 yrs) controls underwent a standard hyperglycemic clamp and a placebo clamp (saline infusion) in a randomized and blinded manner with a two-week interval. Working, semantic, and episodic memory was tested at both clamps after 110 minutes by a nurse blinded to the clamp and the glycemic status of the participant. The results of the memory tests were standardized (z-scores). All participants were free from cognitive disorders, depression, CVD, or medication influencing their memory.

**Results:** T2D patients had a higher body mass index, BMI, than controls (27.9±3.5 vs. 25.5±2.6 kg/m<sup>2</sup>,  $p=0.002$ ) but there was no difference in blood pressure or educational levels. Controls had better working memory (z-score 0.26±0.79) in the placebo clamp compared to T2D (z-score -0.25±0.74,  $p=0.007$ ) while there were no differences in episodic or semantic memory. Women with T2D had poorer working memory than controls in the placebo clamp even after adjustment for BMI, fasting glucose and glucose at time for memory testing (B -0.60, SE 0.27,  $p=0.037$ ). In the hyperglycaemic clamp the working memory decreased in controls and improved in T2D which equated the difference (controls z-score 0.16±0.81 vs. T2D -0.15±0.69,  $p=0.08$ ). This pattern was driven by changes among women (placebo clamp: controls working memory 0.47±0.74 vs. T2D -0.21±0.64,  $p=0.007$ ; hyperglycaemic clamp: controls working memory 0.45±0.88 vs. T2D -0.05±0.76,  $p=0.08$ ). The majority of women in the control group (71%) impaired their working memory during the hyperglycaemic clamp compared to the placebo clamp. The opposite pattern was observed among T2D women with 61% improving their working memory in the hyperglycaemic clamp compared to the placebo clamp.

**Conclusion:** Women with T2D have a poorer working memory compared to non-diabetic women during normoglycaemic conditions. In acute hyperglycaemia the two groups show different reactions equating the difference. This suggests altered prefrontal function in women with T2D with a decreased plasticity to a metabolic change.

Supported by: Västerbotten County Council

### 15

#### Insulin therapy in patients with type 2 diabetes mellitus during the first 24 hours of acute stroke: advantages of intravenous infusion

L.G. Strongin, I.G. Grigoryan, N.G. Belyaeva, A.V. Gustov;  
Medical Faculty, State Medical Academy, Nizhny Novgorod, Russian Federation.

**Background and aims:** Points of intravenous infusion for blood glucose control in patients with stroke and type 2 diabetes mellitus is proved at a target level less than 7 mmol/l, but for the more acceptable level 7.8-10mmol/l it is not so obvious. The comparison of the efficiency and safety of intravenous infusion with fractional subcutaneous injections was carried out in the given research.

**Materials and methods:** 73 patients with stroke and type 2 diabetes mellitus were included in the given research. Patients were subdivided in to 2 groups. In the basal group (n=36) the continuous infusion of insulin within 24 hours after the development of stroke was spent. Patients in the group of comparison (n=37) received insulin therapy in the form of subcutaneous injections. There were no differences in the baseline characteristics between groups. In both groups short-acting insulin was titrated according to glucose levels to achieve the blood glucose level between 7.8 and 10 mmol/l. The effect of therapy was estimated according to mortality and dynamics of symptoms



on National Institutes of Health Stroke Scale and Barthel Activities of Daily Living Index,

**Results:** In the basal group target glucose levels have been reached through 2(2-3)hour, in the control group in 5(3-6) hour,  $p=0.0019$ . In the group of insulin infusion 97% patients achieved the glucose targets of the study protocol, versus 71% in the control group, ( $p=0.012$ ). The mean daily glycemia was 8,7 (7,5-11,3) mmol/l in the group of infusion and 9,7(7-11,7) mmol/l in the group of comparison,  $p=0.025$ , amplitude of fluctuations of glycemia within a day was 0,95 mmol/l in the basal group and 5,3 mmol/l in the control group,  $p<0.01$ , frequency of hypoglycemia in the group of intravenous infusion was 9%, in the group of subcutaneous injections 22%,  $p=0.037$ . The total amount of insulin used within the day in the basal group was 21(13,5-43) units, in the group of comparison 48 (16-66) units,  $p<0.01$ . There were no differences in hospital mortality between the groups: in the group of intravenous infusion 25% patients died, in the group of comparison 32,4%,  $p=0.32$ . Dynamics of neurologic deficiency according to the National Institutes of Health Stroke Scale was 12 points in the basal group versus 6 points in the control group,  $p<0.01$ . The difference on Barthel Activities of Daily Living index was 45 points in the infusion group and 20 points in the control group,  $p<0.01$ .

**Conclusion:** Glucose control using continuous intravenous infusion at a target glucose level between 7,8-10 mmol/l has advantages concerning efficiency and safety over subcutaneous injections of insulin in the acute period of stroke in patients with diabetes mellitus.

## 16

**The significance of social non-amendable factors in the two-year functional outcome of ischaemic stroke male and female diabetic patients**  
K. Tzirogiannis<sup>1</sup>, V. Dragoumanos<sup>1</sup>, N. Melas<sup>2</sup>, M. Vourvou<sup>3</sup>, E. Foustieris<sup>1</sup>, A. Melidonis<sup>1</sup>;

<sup>1</sup>Diabetes Center, 'Tzanio' General Hospital, Piraeus, <sup>2</sup>1st Department of Internal Medicine, 'Tzanio' General Hospital, Piraeus, <sup>3</sup>Intensive Care Unit, 'Papageorgiou' General Hospital, Thessaloniki, Greece.

**Background and aims:** Diabetes doubles the risk of stroke recurrence and aggravates the prognosis of stroke patients. The multifactorial, regardless of gender nature of stroke etiology is also well established. Based on their susceptibility to modification, stroke risk factors are classified as amendable or non-amendable. The purpose of the present study is to evaluate and compare the effect of various non-amendable social risk factors on the two-year functional outcome of ischemic stroke (IS) male and female diabetic patients.

**Materials and methods:** The study population consisted of 312 type II Caucasian diabetic patients that suffered primary IS. Assessment was performed independently by three neurology specialists upon hospital admission and at two years after the initial episode according to NIHSS (0-42). Improvement of functional outcome was considered a decrease of at least 4 units at NIHSS. Comparisons between categorical variables were evaluated by Fisher's exact test.

**Results:** After 24 months 175 out of 312 (56.1%), 75 (24%) male and 100 (32.1%) female patients presented neurological improvement. Female patients (OR=1.81,  $p=0.009$ ), patients that cohabited with their partners (OR=2.35,  $p<0.001$ ), their children (OR=4.46,  $p<0.001$ ), or both (OR=4.61,  $p=0.001$ ) exhibited significantly better post stroke neurological outcome at two years. Divorced (OR=0.24,  $p=0.027$ ), single (OR=0.09,  $p<0.001$ ), childless (OR=0.14,  $p<0.001$ ) or single living patients (OR=0.16,  $p<0.001$ ) exhibited lower rates of neurological improvement. Contrary to male, female patients exhibited better prognosis if cohabited with partner and children (OR=4.03,  $p=0.01$ ), showed no correlation if childless (OR=0.62,  $p=0.169$ ), and worsened if divorced (OR=0.06,  $p=0.001$ ).

**Conclusion:** Married status and cohabitation with children are among the non-amendable social risk factors that confer a better two-year functional outcome in diabetic IS patients. Being single, living alone, and childlessness is connected with deteriorated post stroke prognosis. Contrary to male, female patients exhibit better neurological outcome if cohabiting with partner and children, exhibit no further improvement if childless, and worsen if divorced.

Two-year post stroke neurological improvement according to NIHSS

	Males		Females		Total	
Non amendable risk factors	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Gender (male)	N/A		N/A		0.55 (0.35-0.86)	0.009
Gender (female)	N/A		N/A		1.81 (1.15-2.85)	0.009
Live alone	0.18 (0.08-0.4)	<0.001	0.16 (0.06-0.38)	<0.001	0.16 (0.09-0.29)	<0.001
Cohabite with partner	2.91 (1.49-5.71)	0.001	1.99 (1.03-3.84)	0.039	2.35 (1.48-3.73)	<0.001
Cohabite with children	5.31 (1.44 - 19.47)	0.006	3.68 (1.74 - 7.77)	<0.001	4.46 (2.4 - 8.29)	<0.001
Cohabite with partner and children	3.25 (0.33-31.95)	0.286	4.03 (1.31-12.32)	0.01	4.61 (1.72-12.34)	0.001
Childless	0.02 (0.01-0.06)	<0.001	0.62 (0.31-1.22)	0.169	0.14 (0.08-0.24)	<0.001
Single	0.15 (0.06-0.35)	<0.001	0.03 (0.01-0.24)	<0.001	0.09 (0.04-0.21)	<0.001
Divorced	2.13 (0.19-24.07)	0.53	0.06 (0.01-0.51)	0.001	0.24 (0.06-0.93)	0.027
Widowed	2.54 (0.83-7.71)	0.09	1.28 (0.49-3.36)	0.608	1.81 (0.88-3.75)	0.102

## 17

**Diabetes mellitus type 2 and markers for stroke: 10 years, prospective study of the 1,334 cases representative cohort**

J. Taton, P. Luźniak, A. Czech, A. Wojciechowska-Luźniak;  
Chair and Department of Internal Diseases and Diabetology, Warsaw Medical University, Poland.

**Background and aims:** The morbidity and mortality due to stroke in persons with type 2 diabetes mellitus is 3-4 times higher than in the general population. Actual epidemiological studies underline the increasing tendency of these indices. The efficacy of the prevention of stroke stands up as the "hot" problem in diabetes mellitus care. It has motivated us to examine the risk of stroke in a well defined, large cohort of type 2 diabetics using a prospective study plan lasting 10 years. The specific aims of the study were : 1) determination of the incidence and mortality due to stroke in a dynamic, comparative way during 10 years , and 2) recognition of the appearance during the 10 years period of risk factors for stroke.

**Materials and methods:** The cohort of 1,334 type 2 diabetes mellitus patients was organized by allocating the patients attending the same outpatient diabetic clinic suitable for prolonged observation. Among patients were 597 males and 737 women; their average age was  $62.61 \pm 10.31$  and the diabetes mellitus duration  $9.40 \pm 6.26$  years. The main clinical determinants such as BMI, blood pressure, fasting and postprandial glycemia, cholesterol, triglycerides, creatinine, albuminuria, and also the co-existing complications of diabetes mellitus type 2 and co-morbid states, were first established at the time of the patients' entry to the study and assessed every year in the 10 years period. The clinical and laboratory parameters of the cardiac and neurological function were specifically evaluated. The morbidity and mortality due to stroke were determined and correlated to the potential risk factors every year separately and as a cumulative value for the whole 10-year period.

**Results:** At the beginning of the study a previous stroke history was found in 62 persons - 34 females and 28 males. The initial prevalence of stroke was 4.6%. In the 10-year period, 135 new episodes of stroke were observed, that is, 7.5%. The cumulative incidence of stroke was equal to 12.1%, that is, 10.8 cases per 1,000 patient - years. The mortality index due to stroke was 11.0%. The significance of the selected clinical risk factors was subjected to multivariate statistical analysis. The risk factors with statistically valid, pathogenic significance were: 1.) age (RR- 1.05, 95% CI 1.03 - 1.07;  $p<0.001$ ), 2.) fasting glycemia (RR- 1.99, 95% CI 1.17 - 3.39;  $p<0.05$ ), 3.) daily albuminuria (RR- 1.98, 95, 95% CI 1.02 - 4.06;  $p<0.05$ ), 4.) atrial fibrillation (RR- 2.91, 95% CI 1.39 - 6.09;  $p<0.01$ ) and 5.) smoking (RR- 1.97, 95% CI 1.17 - 3.00;  $p<0.01$ ).

**Conclusion:** Among 1,334 cases of the studied cohort of type 2 diabetes mellitus observed during the 10-year period in the same outpatient clinic, the cumulative stroke incidence was 12.1%, that is, 10.8 of cases per 1,000 person-years. The cumulative mortality reached the level of 11.0%. The main objectively established clinical risk factors for the stroke were age, of patients fasting glycemia, daily albuminuria, atrial fibrillation and smoking. This information should be taken under consideration in establishing an individual plan of stroke prevention.

## 18

**Effects of type 2 diabetes on 12-year cognitive change: results from the MAAS prospective cohort study**P.J.J. Spauwen<sup>1</sup>, S. Köhler<sup>1</sup>, F.R.J. Verhey<sup>1</sup>, C.D.A. Stehouwer<sup>2</sup>, M.P.J. van Boxtel<sup>1</sup><sup>1</sup>Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNS), <sup>2</sup>Internal Medicine, Cardiovascular Research Institute (CARIM), Maastricht, Netherlands.

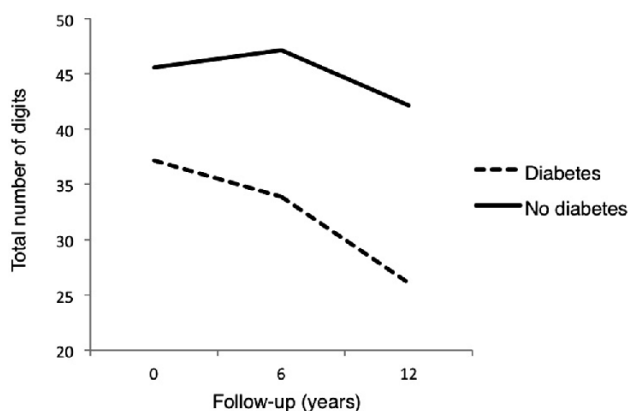
**Background and aims:** Type 2 diabetes is associated with an increased risk of cognitive impairment and dementia. Despite this increased risk, results from studies into the cognitive trajectories of patients with type 2 diabetes have been inconclusive. The aim of the present study was to investigate the effect of baseline and incident type 2 diabetes on change in cognitive performance over 12 years.

**Materials and methods:** A sample of 1,290 individuals aged 40 years and older at baseline, participating in the Maastricht Aging Study (MAAS), were cognitively tested at baseline, after six years, and after 12 years. Of these, 68 participants had type 2 diabetes at baseline, and 54 and 57 had incident diabetes at 6-year and 12-year follow-up, respectively. Changes in performance on tests of information processing speed, executive function, and verbal memory from baseline to 6-year and to 12-year follow-up were compared between groups. Effects of diabetes on cognitive decline were adjusted for demographic variables, history of smoking, alcohol intake, and comorbid conditions including hypertension, cardiovascular disease, body mass index, and depression.

**Results:** Linear mixed model analysis was used to test effects of baseline and incident diabetes on cognitive decline. Participants with baseline diabetes showed a larger decline in information processing speed (estimate, -7.64, 95% confidence interval, -10.47 to -4.81), in executive function (21.82, 13.57 to 30.06), and in delayed word recall (-1.35, -2.50 to -0.20) over 12-year follow-up compared with controls, after adjustment for demographic variables, history of smoking, alcohol intake and comorbid conditions. No significant difference in decline was observed for immediate word recall (-1.88, -5.28 to 1.52). Interestingly, participants with incident diabetes did not show a larger decline in any cognitive domain compared with controls.

**Conclusion:** This study clearly indicates that patients with baseline type 2 diabetes show accelerated cognitive decline compared with individuals without diabetes, while patients with incident diabetes do not yet show this decline. This might provide a window of opportunity for prevention and early treatment of diabetes related cognitive deficits. For this, it is important to assess cognitive status at an early stage of the disease and to repeat cognitive assessments on a regular basis.

**Figure 2 | Adjusted effect of type 2 diabetes on change in information processing speed from baseline to 6-year and to 12-year follow-up**



## OP 04 Insulin action in the liver

## 19

**In vivo mechanisms of saturated and unsaturated fat-induced hepatic insulin resistance**T. Galbo<sup>1</sup>, T. Alves<sup>1</sup>, M. Kahn<sup>1</sup>, D. Zhang<sup>1,2</sup>, V.T. Samuel<sup>1,2</sup>, G.I. Shulman<sup>1,2</sup><sup>1</sup>Internal Medicine and Endocrinology, Yale University School of Medicine, USA, <sup>2</sup>Howard Hughes Medical Institute, New Haven, USA.

**Background and aims:** Ectopic lipid accumulation in the liver is strongly associated with hepatic insulin resistance, however, the cellular mechanism by which intracellular lipid-induces hepatic insulin resistance is controversial. It has been hypothesized that saturated (sat) and unsaturated (unsat) fatty acids (FAs) cause hepatic insulin resistance through divergent mechanisms, where sat FAs cause insulin resistance through activation of a TLR pathway leading to activation of Protein Phosphatase 2A (PP2A) and inhibition of insulin signalling at the level of AKT2 phosphorylation, whereas unsat FAs lead to accumulation of diacylglycerols (DAGs) leading to activation of PKCε leading to reduced insulin signalling at the level of the insulin receptor kinase. In this study we examined this hypothesis by directly comparing the effects of sat- and unsat-fat diet on hepatic insulin signalling in awake rats.

**Materials and methods:** Male S-D rats were fed either a chow diet or a high-fat diet (60% calories from fat) based on lard (sat) or safflower (unsat) for three days to specifically induce hepatic insulin resistance. Basal parameters were determined in one set of rats (n=10 per group) while separate groups of rats were infused with insulin to assess the effects of the different diets on insulin signalling (n=6 per group). Statistical significance was determined by ANOVA followed by post hoc t-test.

**Results:** Rats fed either a sat or unsat high-fat diet manifested increased plasma FAs (~50 and 100%, P<0.05 and P<0.001 respectively), liver triglycerides (~2- and 3-fold, P<0.05 and P<0.001) and DAGs (both ~3-fold, P<0.05 and P<0.01) compared to the chow fed rats. Furthermore, both sat and unsat high-fat diets were associated with a ~5-fold increase in activation of PKCε (P<0.05) and an ensuing reduction in insulin-stimulation of IRS1 and IRS2 tyrosine phosphorylation (~50-70%, P<0.05). Both sat and unsat diets led to a relatively small ~20% (P<0.05 and P<0.01) increase in activity of the Akt-inactivating PP2A. Furthermore both sat and unsat diets resulted in a ~50% reduction in insulin-stimulated phosphorylation of the nuclear Akt2 substrate and transcriptional inducer of gluconeogenic gene expression, FoxO1 (both P<0.05). Consistent with this, we found an impairment of insulin-stimulation of Akt2 translocation to the plasma membrane (~30%, P<0.05) and nucleus (~50%, P<0.05), and a ~40% (P<0.01) decrease in insulin-stimulation of nuclear levels of phosphorylated, active Akt2 in animals fed either sat or unsat diets.

**Conclusion:** Taken together our data suggest that saturated and unsaturated fatty acids promote hepatic insulin resistance through a similar mechanism involving DAG activation of PKCε resulting in decreased insulin stimulation of IRS-1 and IRS-2 tyrosine phosphorylation and possibly through activation of PP2A.

Supported by: NIH DK40936

## 20

**Induction of a hepatic lipid droplet protein induces a hepatic steatosis without insulin resistance**K. Minehira<sup>1</sup>, T. Bouduban<sup>1</sup>, P. Gual<sup>2</sup>, A. Tran<sup>2</sup>, S. Bonnafant<sup>2</sup>, F. Mange<sup>1</sup>, G. Willemin<sup>1</sup><sup>1</sup>Department of Physiology, University of Lausanne, Switzerland, <sup>2</sup>Equipe 8 complications hépatiques de l'obésité, INSERM, U895, Nice, France.

**Background and aims:** It has been believed that an abnormal lipid accumulation in tissues, such as a hepatic steatosis, induces insulin resistance via lipid intermediates. In hepatocyte, neutral lipids are stored inside of lipid droplets which consist of a phospholipid monolayer and lipid droplet proteins such as perilipins on their surface. Any degradation of the neutral lipids from lipid droplets could contribute to the accumulation of lipid intermediates. However whether the lipid droplet proteins are implicated in the development of hepatic insulin resistance has not been understood. We hypothesized that these lipid droplet proteins increase a capacity of hepatocytes to store lipids and protect against the insulin resistance.

**Materials and methods:** We studied the effect of perilipin 5 (Plin5) in mouse hepatocytes (AML-12 cells) by overexpressing with an adenoviral vector, or

by downregulating with a siRNA technique. Triglyceride content and the insulin sensitivity were measured by a chemical analysis and by a western blotting respectively. In addition, we studied the effect of Plin5 overexpression in mice and checked a degree of hepatic steatosis and a presence of insulin resistance. In order to understand their roles in human, we studied the expression of Plin5 in the liver biopsies from patients with diverse hepatic steatosis. We also compared the expression in the liver from patients with or without diabetes.

**Results:** Overexpression of Plin5 significantly induced triglycerides accumulation compared to the control cells. On the contrary, the downregulation resulted in the formation of abnormal fractionated lipid droplets in AML-12 cells. The hepatic steatosis induced by the overexpression of Plin5 was dissociated from insulin resistance, however the downregulation of Plin5 strongly induced insulin resistance in AML-12 cells. As found in in-vitro studies, mice overexpressing Plin5 in the liver developed a severe hepatic steatosis. Their insulin sensitivity was intact judged by a pyruvate tolerance test and by a phosphorylation of Akt upon an insulin injection. In human liver biopsies, the expression of Plin5 was higher in patients with severe hepatic steatosis. Interestingly, once the patients developed diabetes, this induction of Plin5 was not observed any more.

**Conclusion:** Our in-vitro and in-vivo studies clearly demonstrated that the induction of Plin5 augmented the capacity of hepatocytes to store triglycerides. Despite this induction of hepatic steatosis, these cells/livers remained insulin sensitive, supporting our hypothesis of the protective role of Plin5 against insulin resistance. In line with the concept, when Plin5 was downregulated, cells became insulin resistant. Together with the data from the human diabetic liver which presented low Plin5 expression, we believe that the Plin5 might protect the fatty liver against the development of insulin resistance by increasing the capacity to store neutral lipids in hepatocytes.

*Supported by: FNS, SFD, SSED, FRRD and Novartis*

## 21

**Proteomic analysis of livers from fat-fed PKCε and PKCδ KO mice: insights into the modulation hepatic lipid metabolism as well as insulin action by PKC isoforms**

C. Schmitz-Peiffer<sup>1</sup>, K. Raddatz<sup>1,2</sup>, B.-Q.M. Liao<sup>1</sup>, B. Diakanastasis<sup>1</sup>;

<sup>1</sup>Diabetes and Obesity Program, Garvan Institute of Medical Research, Sydney, Australia, <sup>2</sup>ZIK Functional Genomics, University of Greifswald, Germany.

**Background and aims:** Specific lipid intermediates, including diacylglycerols (DAG), play roles in the inhibition of insulin action. Protein kinase C (PKC) isoforms are activated by DAG, and we have shown that deletion of either of PKCδ or PKCε improves glucose tolerance in fat-fed mice. We also demonstrated that these PKC isoforms play unexpected roles in the modulation of hepatic lipid metabolism itself. PKCδ-deficient mice show reduced triglyceride (TG) accumulation in the liver whereas PKCε-deficient mice exhibit increased TG storage. These models were therefore employed in proteomic and physiological experiments in order to gain insights into the regulation of lipid metabolism and insulin action in the liver.

**Materials and methods:** We performed quantitative proteomic analyses of livers from chow- and fat-fed wildtype (WT) and PKCδ and PKCε KO mice, using an in vivo adaptation of stable isotope labelling with amino acids in cell culture (SILAC) methodology. We measured ketone bodies and glucose in blood from fed and fasted WT and PKCε KO mice. We employed isolated primary hepatocytes from these animals to examine lipid metabolism using tracers.

**Results:** For each PKC, the expression ratios of approximately 3000 proteins were determined in the 4 groups of animals, in each of two separate experiments. Of these, 23 proteins were upregulated by >1.5-fold in livers from fat-fed PKCδ KO mice, compared to fat-fed WT mice in each experiment, and 6 proteins were downregulated >1.5-fold. Similarly, 18 proteins were upregulated in livers from fat-fed PKCε KO mice, and 17 proteins were downregulated. These included enzymes of lipid, cholesterol and amino acid metabolism, proteins linked to redox stress and also cytoskeletal and nuclear proteins. Certain proteins were affected by both PKC isoforms, either in a similar or a reciprocal manner, consistent with a role either in the improvement in insulin sensitivity or in the isoform-specific effects on TG storage respectively. In particular, enhanced expression of specific oxidoreductases correlated with improved insulin action. In addition, pathway analysis of the sets of altered proteins implicated PKCε in the regulation of cholesterol and ketone body synthesis. In agreement, cholesterol synthesis was found to be elevated by over 2-fold in PKCε KO hepatocytes. Total cholesterol, however,

was not correspondingly increased, suggesting additional effects on its downstream metabolism. Furthermore, in fasted PKCε KO mice, the rise of blood ketone levels was reduced by 40%, whereas blood glucose levels remained 30% higher than those of WT mice.

**Conclusion:** Proteomic analysis of livers from PKC KO mice has generated new avenues for investigation into mechanisms by which these kinases regulate hepatic lipid metabolism, and also how their deletion may lead to an improvement in insulin sensitivity in fat-fed mice. We have also uncovered a novel phenotype of PKCε KO mice, which exhibit improved glucose homeostasis and reduced ketogenesis upon fasting. Together with the previously reported improvement in insulin action upon fat-feeding, this further promotes PKCε as a key therapeutic target for the treatment of glucose intolerance.

*Supported by: NHMRC and Diabetes Australia Research Trust*

## 22

**Transient increase in mitochondrial oxidative capacity during the development of insulin resistance in a mouse model of non-alcoholic fatty liver**

T. Jelenik<sup>1</sup>, G. Séquaris<sup>1</sup>, J. Szendrödi<sup>1,2</sup>, J. Kotzka<sup>3</sup>, E. Phielix<sup>1</sup>, B. Knebel<sup>3</sup>, P. Nowotny<sup>1</sup>, H.-J. Partke<sup>1</sup>, D. Müller-Wieland<sup>4</sup>, M. Roden<sup>1,2</sup>;

<sup>1</sup>Institute for Clinical Diabetology, German Diabetes Center, Heinrich Heine University, Leibniz Center for Diabetes Research, Düsseldorf, <sup>2</sup>Clinic for Metabolic Diseases, University Clinic Düsseldorf, Heinrich Heine University, <sup>3</sup>Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Heinrich Heine University, Leibniz Center for Diabetes Research, Düsseldorf, <sup>4</sup>Asklepios Clinic St. Georg, Hamburg, Germany.

**Background and aims:** Non-alcoholic fatty liver (NAFL) and ectopic lipid storage in skeletal muscle have been associated with insulin resistance and abnormal mitochondrial function in obesity and type 2 diabetes. The underlying causal relationships are yet unclear. The aim of this study was to determine the role of mitochondrial function during the development of lipid-induced NAFL and insulin resistance.

**Materials and methods:** We examined liver and muscle metabolism in 18- and 36-weeks-old female mice with secondary NAFL due to adipose tissue-specific overexpression of the sterol regulatory-element binding protein-1c (aP2-SREBP-1c: AP2; n=6-7) and in age- and sex-matched wild-type controls on C57BL/6 background (CON; n=6-7). Insulin sensitivity was determined in 36-weeks-old mice by hyperinsulinemic-euglycemic clamps in combination with the isotope glucose technique. Mitochondrial oxidative capacity was measured in permeabilized tissues using high-resolution respirometry (Oroboros Instruments) and mitochondrial content was assessed from the ratio of mitochondrial DNA to nuclear DNA, using quantitative PCR.

**Results:** Mean liver weight was 37% and 86% higher (p<0.001) in 18-weeks-old and in 36-weeks-old AP2 mice when compared to CON. Mitochondrial oxidative capacity was improved in 18-weeks-old AP2 mice as assessed by 75% greater pyruvate-dependent mitochondrial respiration in liver (p<0.05) and 93% greater fatty acid-dependent mitochondrial respiration in muscle (p<0.05). On the other hand, 36-weeks-old AP2 mice displayed 25% lower (p<0.05) pyruvate-dependent muscle mitochondrial respiration. Mitochondrial content in muscle and liver remained unchanged. Basal endogenous glucose production (EGP) was doubled in AP2 mice at the age of 36-weeks (31±4, CON: 17±2 mg/kg/min; p<0.05). These results were in accordance with higher 6-h fasting serum glucose (216±16, CON: 108±14 mg/dl; p<0.05). Furthermore, 36-weeks-old AP2 mice had 43% lower insulin-mediated glucose disposal in peripheral tissues (33±4, CON: 57±9 mg/kg/min; p<0.05). Insulin suppression of EGP as well as serum non-esterified fatty acids was not affected by the AP2 genotype.

**Conclusion:** Initially, hepatic steatosis transiently increases oxidative capacity to compensate for elevated energy supply. However, later stages of NAFL are associated with decreased oxidative capacity and impaired insulin sensitivity in muscle of AP2 mice.

*Supported by: DDG, Menarini, Dr. Schröder Foundation*



## 23

### Comparison between intraperitoneal and subcutaneous insulin infusion: link between routes of administration and hepatic oxidative stress

S. Ros<sup>1</sup>, S. Sigrist<sup>1</sup>, E. Seyfritz<sup>1</sup>, W. Bietiger<sup>1</sup>, C. Péronet<sup>1</sup>, M. Pinget<sup>2,3</sup>, N. Jeandier<sup>2</sup>;

<sup>1</sup>Centre Européen d'Etude du Diabète, <sup>2</sup>Service d'Endocrinologie, Diabète, Maladies Métaboliques, <sup>3</sup>Université de Strasbourg, Strasbourg, France.

**Background and aims:** Subcutaneous insulin infusion (CSII) is nowadays the gold standard for type 1 diabetic patient therapy. Continuous Intraperitoneal Insulin Infusion (CPII), in contrast to peripheral SC route, allows physiological portal insulin administration, first hepatic bypass and partial normalization of hepatic metabolism. However, its impact on oxidative stress (OS), leading to chronic complications is not known. The aim of this study was to compare metabolic and oxidative parameters in these two modes of insulin administration, at the same dose, in diabetic rats.

**Materials and methods:** Diabetic Wistar rats (streptozotocin, 100mg/kg) were randomized into 3 groups: diabetic rats with no insulin, with Insuplant® (7UI/200g/j) in Alzet osmotic mini-pump in CSII or CPII during 1 (W<sub>1</sub>) or 4 weeks (W<sub>4</sub>), compared to age match-controls. Blood glucose, fructosamine, IGF-1, total antioxidant capacity (TAOC) and lipid peroxides were determined in plasma. Liver and mesenteric artery OS were assessed by the probe dihydroethidine and liver glycogen and apoptosis were quantified. Redox-sensitive pathways implicated in glucose metabolism and glucocorticoid receptor (GR) were determined by Western blotting.

**Results:** In addition to better fructosamine (W<sub>4</sub>: p=.006) and limitation of weight loss with CPII (after 20 days: p=.016, W<sub>4</sub>: p=.006) in equal doses, CPII specifically protected the liver from diabetes induced-OS, in comparison to vessels OS, increased the expression of catalase and superoxide dismutase (antioxidant enzymes) in the liver. Both insulin treatments normalized p67phox, a subunit of NADPH oxidase (antioxidant enzymes) and protected against the activation of p38 (redox-sensitive kinase) in the liver, ensuring the maintenance of an intermediary TAOC between non-diabetic and diabetic-rats and protecting against lipid peroxidation. Both insulin treatments protected against the beginning (W<sub>4</sub>) of liver apoptosis observed in diabetic rats. CPII decreased density of diabetes induced-liver macrophages, but have no effect on systemic inflammation associated with diabetes (α2-macroglobulin). From W<sub>1</sub>, CPII allowed the restoration of liver glycogen storage and preserved the phospho-akt/glucose synthase kinase 3 pathway implicated in glycogen synthesis. Better release of IGF-1 was observed from W<sub>1</sub> during CPII and preferably but not significantly at W<sub>4</sub>. Moreover, all these beneficial effects of CPII on liver were associated with an upregulation of GR expression from W<sub>1</sub>.

**Conclusion:** CPII induced better glycaemic control with the same insulin doses than CSII. Hepatic oxidative stress and its consequences associated with diabetes are prevented by CPII, including the fine regulation of the anti-/pro-oxidant enzymes balance. CPII provided a better release of IGF-1 and a restocking of hepatic glucose by preserving in part the liver metabolic pathway. All these results could play a role in improving glycaemic fluctuations and demonstrated the importance of focusing on the first hepatic bypass of insulin, all for the limitation/prevention of diabetic complications.

Supported by: "Vaincre le Diabète"

## 24

### Novel PEGylated basal insulin LY2605541 has a preferential hepatic effect on glucose metabolism

M.C. Moore<sup>1</sup>, M.S. Smith<sup>1</sup>, K.F. Mace<sup>2</sup>, V.P. Sinha<sup>2</sup>, M.D. Michael<sup>2</sup>, S.J. Jacober<sup>2</sup>, J.M. Beals<sup>2</sup>, A.D. Cherrington<sup>1</sup>;

<sup>1</sup>Molecular Physiology & Biophysics, Vanderbilt University, Nashville, <sup>2</sup>Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, USA.

**Background and aims:** LY2605541 (LY) is insulin lispro with a 20 kDa polyethylene glycol (PEG) moiety covalently attached to lysine B28. LY is designed to have a large hydrodynamic size that slows absorption and reduces clearance, resulting in a longer duration of action. To assess the impact of PEGylation on the pharmacodynamics of insulin lispro, LY was compared to regular insulin in vivo.

**Materials and methods:** Euglycemic clamps were carried out in conscious dogs. The studies were initiated with a primed, continuous infusion of [3-<sup>3</sup>H]glucose via peripheral vein (Pe). After 90 minutes of tracer equilibration, there was a 30-minute basal period and a 5-h clamp period. Infusions during the clamp period were: Pe regular insulin (0.3 mU/kg/minute; insulin control

[CONTROL], n=6) or LY (0.5 nmol/kg bolus then 0.5 nmol/kg/h, n=6); Pe somatostatin; intraportal glucagon (basal replacement); and Pe glucose.

**Results:** Both groups exhibited basal net hepatic glucose output at 1.9 mg/kg/minute. In CONTROL, this gradually fell to 0, but in LY the liver switched to net uptake within 30 min, reaching a rate of 1.1±0.2 mg/kg/minute during the last hour (CONTROL vs. LY, P<0.05). At the end of the clamp, glucose rate of appearance (R<sub>a</sub>) was suppressed by 1.6±0.3 and 2.3±0.2 mg/kg/minute in CONTROL and LY, respectively (P<0.05), and unidirectional hepatic glucose uptake was 33% higher in LY than in CONTROL (P=0.06). Non-hepatic glucose uptake in LY increased less than in CONTROL (last h: 0.7±0.3 vs. 2.0±0.8 mg/kg/minute above basal). The glucose infusion rates (3.9±0.9 and 3.9±0.6 mg/kg/minute) and glucose rate of disappearance (R<sub>d</sub>; 5.2±0.8 and 4.6±0.7 mg/kg/minute) were not significantly different during the final hour in CONTROL and LY, respectively. Arterial glycerol and nonesterified fatty acid levels fell in CONTROL but initially increased in LY, thereafter falling and converging with those in CONTROL near the end of the study.

**Conclusion:** These data demonstrate that LY2605541 has a preferential hepatic effect similar to endogenously produced insulin and different from exogenously administered human insulin.

Supported by: Eli Lilly & Co

## OP 05 New modulators of energy expenditure

25

### Fibroblast growth factor 21 (FGF21) increases energy expenditure in a leptin-dependent manner

T. Coskun, A.C. Adams, S.L. DuBois, L.S. O'Farrell, C. Cheng, A. Kharitonkov;  
Endocrine, Eli Lilly and Company, Indianapolis, USA.

**Background and aims:** FGF21 regulates whole body metabolism through distinct actions on the liver, white and brown adipose tissues. Previously, we have demonstrated that FGF21 corrects obesity in part via increasing energy expenditure. However, the mechanism of action mediating this increase is poorly understood. In earlier studies, we observed that chronic FGF21 treatment up-regulated leptin receptor gene expression in liver and white adipose tissue. Interestingly, while receptor expression was increased FGF21 administration caused a significant reduction in plasma leptin levels suggesting FGF21 may act in part via leptin sensitization. In series of *in vivo* studies, we aimed to investigate the interaction between FGF21 and leptin with regards to the regulation of body weight and energy expenditure.

**Materials and methods:** Lean, diet-induced obese or ob/ob mice were chronically treated with FGF21, leptin, or a combination of both leptin and FGF21 and body weight, food intake, and energy expenditure were measured.

**Results:** Our results demonstrated that subcutaneous leptin or FGF21 infusion decreased body weight in a dose responsive manner in lean C57/BL6 mice. In DIO mice, leptin infusion alone (up to 100 µg/mouse/day) did not cause any BW reduction, however, FGF21 treatment caused even greater body weight reduction in DIO mice than we observed in lean mice. Importantly, treatment with a combination of leptin in addition to FGF21 restored leptin sensitivity in DIO mice with the combination demonstrating increased efficacy when compared to either agent alone or the summation of the two suggesting synergistic effects. In ob/ob mice FGF21 led to a modest reduction in body weight, however, this reduction was significantly blunted when compared to the magnitude of the effect observed in DIO mice. Furthermore, in the ob/ob mice the energy expenditure effect of FGF21 was largely absent suggesting leptin is required for FGF21 to modulate metabolic rate. Supporting this hypothesis we demonstrate that replacement of leptin in ob/ob mice restores the FGF21 effect on energy expenditure.

**Conclusion:** Therefore, we conclude that FGF21 is able to rapidly and robustly increase leptin sensitivity in states of diet induced obesity and may provide additional avenues for pharmacological intervention with this protein.

26

### Metabolic effects of mitochondrial uncoupling in murine skeletal muscle: essential role of AMP-activated protein kinase in metabolic improvements of UCP1-transgenic mice?

M. Ost, A. Voigt, S. Keipert, J. Dokas, S. Klaus;  
Department of Pharmacology/ Group of Energy Metabolism, German Institute of Human Nutrition, Nuthetal, Germany.

**Background and aim:** Altering the efficiency of mitochondrial respiration may represent an attractive target for the treatment of obesity as well as related disorders. Transgenic (UCP1-TG) mice with ectopic expression of UCP1 in skeletal muscle (SM) show a phenotype of increased energy expenditure and improved glucose tolerance counteracting most of the detrimental effects of high fat diet. We could show an increased AMPK phosphorylation in SM of UCP1-TG mice, likely due to the energy depletion through uncoupling by UCP1. However, the full molecular mechanisms leading to the ameliorated metabolic phenotype of UCP1-TG mice are still not known. The aim of this study was to investigate the potential role of skeletal muscle AMPK activation in the healthy metabolic phenotype of UCP1-TG mice.

**Material and methods:** We generated double transgenic (DTG) mice, by crossing of UCP1-TG mice with DN-AMPK mice overexpressing a dominant negative  $\alpha 2$  subunit of AMPK in SM which results in decreased AMPK activity. For basal characterization we investigated body composition, energy metabolism, glucose homeostasis and physical activity under standard chow diet in young male & female wild-type (WT) and TG mice (DN-AMPK, UCP1-TG, DTG). At 12 weeks of age mice were killed for analysis of substrate metabolism in SM (AMPK activity, *ex vivo* glucose uptake).

**Results:** Skeletal muscle AMPK activity was assayed *in vitro* by incorporation of  $^{32}\text{P}$  into a synthetic SAMS peptide after immunoprecipitation of AMPK $\alpha$  subunits. As expected, AMPK $\alpha 2$  activity was increased by  $50 \pm 20\%$  ( $p < 0.01$ ) in SM of UCP1-TG mice, whereas in DN-AMPK and DTG mice AMPK $\alpha 2$  activity was impaired by  $90 \pm 9\%$  ( $p < 0.001$ ). AMPK $\alpha 1$  activity was not affected in any genotype. Biometric analysis showed decreased body weight, lean, and fat mass for both UCP1-TG and DTG compared to WT and DN-AMPK mice. DTG mice showed slightly reduced body weight and significant decreased body length ( $p < 0.001$ ) compared to UCP1-TG mice, but no differences in body composition. Energy intake and weight-specific total energy expenditure were increased, both in UCP1-TG and DTG mice. Moreover, weight-specific resting energy expenditure was increased in DTG mice only ( $p < 0.001$ ). In addition, voluntary physical activity during night was decreased in DTG mice ( $p < 0.05$ ). Basal glucose uptake in EDL muscle *ex vivo* was significantly increased in UCP1-TG ( $p < 0.01$ ), but not in DTG mice compared to WT. However, maximum insulin stimulated glucose uptake was similar in all genotypes. No gender differences were observed.

**Conclusion:** Collectively, the first basal characterization shows that the reduction of AMPK $\alpha 2$  activity in SM slightly affects biometrics, energy metabolism and physical activity of young DTG compared to UCP1-TG mice. *In vivo* metabolic challenges (dietary intervention, exercise endurance) and further *ex vivo/ in vitro* pathway analysis will follow to elucidate the role and significance of AMPK on energy and substrate metabolism in UCP1-TG mice in more detail.

27

### Conventional knockout of Tbc1d1 in mice results in impaired insulin-stimulated glucose uptake in skeletal muscle but has no impact on whole body glucose and insulin tolerance

A. Chadt<sup>1</sup>, J. Dokas<sup>2</sup>, T. Nolden<sup>3</sup>, H. Himmelbauer<sup>4</sup>, H.-G. Joost<sup>2</sup>, H. Al-Hasani<sup>1</sup>;

<sup>1</sup>Biochemistry, German Diabetes Center (DDZ), Duesseldorf, Germany, <sup>2</sup>Pharmacology, German Institute of Human Nutrition (DIfE), Potsdam-Rehbruecke, Germany, <sup>3</sup>Max Planck Institute for Molecular Genetics, Berlin, Germany, <sup>4</sup>Centre for Genomic Regulation (CRG) and UPF, Barcelona, Spain.

**Background and aims:** We recently demonstrated that the lean and diet-induced obesity-resistant SJL mouse strain carries a loss-of-function mutation in the signalling protein TBC1D1. Introgression of a 10 Mb fragment containing this non-functional allele into a C57BL/6J background (Recombinant congenic strain, RCS) resulted in mice with reduced body weight and body fat. Moreover, the respiratory quotient of these animals was decreased, indicating increased whole body lipid utilization due to the lack of TBC1D1. *Ex vivo* experiments in intact isolated skeletal muscles showed an increase in fatty acid oxidation (FAO) but on the other hand a reduced insulin-stimulated glucose uptake. However, except of the non-functional *Tbc1d1* gene, additional 53 genes from SJL were present in the genome of the RCS mice. Therefore, we generated a conventional *Tbc1d1*-knockout (KO) mouse using the genetrap technology in order to exclusively link the observed phenotype to the mutated *Tbc1d1* gene.

**Material and methods:** Body weight and body composition of mice on a standard diet were regularly monitored. At distinct time points, respiratory quotient as well as energy expenditure was measured and the mice underwent intraperitoneal glucose and insulin tolerance tests. In parallel, we measured blood glucose and plasma insulin values after a period of 16 h fasting and also postprandially. Finally, EDL (*Extensor digitorum longus*) and soleus muscle were dissected and *ex vivo*  $^3\text{H}$ -palmitate oxidation and  $^{14}\text{C}$ -2-DOG (deoxyglucose) uptake were measured.

**Results:** In agreement with our previous results, conventional *Tbc1d1*-knockout mice displayed a reduced body weight. Moreover, these mice also showed a decreased respiratory quotient and an elevated resting metabolic rate (RMR), indicating moderately increased energy expenditure. Also, GLUT4 protein content was decreased by approximately 50% in glycolytic skeletal muscle of *Tbc1d1*-deficient mice whereas expression of the related signaling protein AS160 (TBC1D4) and the glucose transporter GLUT1 were not changed. *Ex vivo* analysis of intact isolated skeletal muscle, revealed that *Tbc1d1* knockout results in enhanced FAO with concomitant decrease of insulin- and AICAR-stimulated glucose transport. Surprisingly, however, there were no gross alterations of whole-body glucose and insulin tolerance due to the lack of TBC1D1 in these mice.

**Conclusion:** Our present results suggest that the SJL-specific mutation in the *Tbc1d1* gene is at least in part responsible for the lean phenotype of SJL mice.

Furthermore, our data demonstrate that TBC1D1 is a critical component for glucose and fatty acid metabolism in skeletal muscle. Whole body glucose and insulin tolerance was not altered indicating compensation of the knockout by the related AS160 protein. Future analysis will clarify how exactly TBC1D1 interferes with GLUT4 protein content in the cell and how a deficiency of this RabGAP protein increases FAO.

Supported by: EFSD/Lilly grant, DDG, DFG

## 28

### Morbid obesity is associated with decreased $\mu$ -opioid receptor binding in the brain reward circuit

H.K. Karlsson<sup>1</sup>, L. Tuominen<sup>1</sup>, J.J. Tuulari<sup>1</sup>, J. Hirvonen<sup>1</sup>, R. Parkkola<sup>2</sup>, P. Nuutila<sup>1</sup>, L. Nummenmaa<sup>1</sup>

<sup>1</sup>Turku PET Centre, <sup>2</sup>Department of Radiology, University of Turku, Finland.

**Background and aims:** Obesity has reached epidemic proportions worldwide, yet little is known about the brain mechanisms that predispose to overeating. Functional imaging studies suggest that overeating might be caused by an imbalance between the reward circuit (including the ventral striatum) and inhibitory networks (including the prefrontal and orbitofrontal cortices). The endogenous opioid system, particularly acting via the  $\mu$ -opioid receptor, mediates reward, and animal studies have suggested that  $\mu$ -opioid receptors are involved in the pathophysiology of obesity. Whether  $\mu$ -opioid receptors are altered in human subjects with obesity is unknown.

**Materials and methods:** To examine whether morbid obesity is associated with changes in brain  $\mu$ -opioid receptor binding we recruited 17 morbidly obese women (mean body mass index [BMI] 41.9, mean age 43) and 9 normal-weight women (mean BMI 23.6, mean age 41). We measured brain  $\mu$ -opioid receptor binding using positron emission tomography (PET) with [<sup>11</sup>C]carfentanil, a selective radioligand for  $\mu$ -opioid receptors. Radioactivity was measured with the GE Healthcare Discovery 690 PET/CT scanner for 51 min, and receptor binding was expressed in terms of BPND, which is the ratio of specific to non-displaceable binding in brain. BPND was calculated for each voxel using the simplified reference tissue model (SRTM) with occipital cortex as the reference region, and parametric BPND maps were compared between the groups using ROI analysis and full-volume statistical parametric mapping with SPM8.

**Results:** Morbidly obese patients had on average 37% lower [<sup>11</sup>C]carfentanil BPND than did control subjects in several brain regions relevant for reward processing in brain, including ventral and dorsal caudate nucleus. Full-volume analysis revealed that BMI correlated negatively with BPND in multiple components of the reward circuit including thalamus, caudate nucleus, amygdala and anterior cingulate cortex, further suggesting a direct link between low  $\mu$ -opioid binding and obesity.

**Conclusion:** We found decreased  $\mu$ -opioid binding in morbidly obese patients in several brain regions implicated in reward processing. Low  $\mu$ -opioid tone may represent a neural substrate for decreased hedonic responses to eating in obesity, and could thus make these individual susceptible to overeating in order to gain the desired hedonic response. Identifying the brain mechanisms involved in overeating is critical for developing new psychological and pharmacological treatments to curb the obesity epidemic. In the next phase of the study, we will examine whether these changes are reversible after bariatric surgery for obesity. Whether decreased  $\mu$ -opioid tone is a cause or consequence of obesity remains to be determined.

Clinical Trial Registration Number: NCT00793143

Supported by: Academy of Finland, Sigrid Juselius Foundation

## 29

### Altered energy metabolism in skeletal muscle cells derived from morbidly obese subjects

V. Aas<sup>1</sup>, S.S. Bakke<sup>2</sup>, N. Niklolic<sup>2</sup>, J. Hjeltnes<sup>3</sup>, H. Thoresen<sup>2</sup>, A.C. Rustan<sup>2</sup>, <sup>1</sup>Institute of Pharmacy and Biomedical Laboratory Science, Oslo and Akershus University College of Applied Sciences, Oslo, <sup>2</sup>School of Pharmacy, University of Oslo, <sup>3</sup>The Morbid Obesity Center, Vestfold Hospital Trust, Tønsberg, Norway.

**Background and aims:** Obesity is strongly associated with insulin resistance and type 2-diabetes (T2D), and about 80 % of patients with T2D are overweight. A study in 2010 at the Morbid Obesity Center in Tønsberg, Norway, revealed that less than 1 out of 3 morbidly obese subjects (BMI >40 kg/m<sup>2</sup>) have T2D, indicating that many morbidly obese subjects possess certain char-

acteristics that protect them against developing T2D. The aim of the present study was to evaluate whether features of skeletal muscle can explain the different susceptibility to develop T2D.

**Methods:** We examined fatty acid and glucose metabolism, as well as metabolic flexibility in human skeletal muscle cells using radiolabeled precursors. Muscle cell mitochondrial and lipid content were examined by fluorescence staining and protein expression by Western blotting. The cells were derived from lean healthy donors (BMI 23 ± 0.9 kg/m<sup>2</sup>), morbidly obese with normal glucose tolerance (BMI 44 ± 2.0 kg/m<sup>2</sup>) and morbidly obese subjects with established type 2 diabetes (BMI 43 ± 1.5 kg/m<sup>2</sup>).

**Results:** Myotubes from obese donors were clearly insulin resistant, assessed by reduced insulin-stimulated phosphorylation of Akt. A 2-fold increase in fatty acid oxidation was observed in cells established from morbidly obese subjects and a approximately 40 % lower metabolic flexibility (measured as the ability of glucose to suppress oleic acid oxidation) compared to cells from lean subjects. The mitochondrial reserve capacity, assessed by oleic acid and glucose oxidation in presence of the mitochondrial uncoupler FCCP, was significantly higher in myotubes from obese T2D subjects than lean. Triacylglycerol lipolysis was 30 % higher for obese T2D subjects than obese with normal glucose tolerance, and lipid accumulation (measured as cell associated lipids and number of lipid droplets) was about 30 % lower. The mitochondrial content was decreased in myotubes from morbidly obese T2D donors compared to morbidly obese with normal glucose tolerance. Glucose uptake and oxidation was not different between the 3 groups.

**Conclusion:** The myotubes from morbidly obese donors maintain altered fatty acid metabolism in culture, and seem to rely more on fatty acid oxidation and are less flexible than myotubes from lean donors. Our data further indicate that lipolysis and mitochondrial capacity may be important for development of T2D in morbidly obese subjects.

## 30

### Regulation of hypothalamic POMC expression by MECP2 and its contribution to leptin resistance

X. Wang, W. Han;

Laboratory of Metabolic Medicine, Singapore Bioimaging Consortium, Agency for Science, Technology and Research (A\*STAR), Singapore.

**Background and aims:** Leptin signals through JAK2/STAT3 pathway to regulate hypothalamic Pomc expression, which is a key regulator of energy and glucose homeostasis. High level of circulating leptin that fails to regulate energy homeostasis is termed as leptin resistance. Leptin resistance can happen at different steps along leptin signaling pathway, including transcriptional regulation of Pomc expression. Maternal or perinatal programming can cause epigenetic changes on Pomc promoter, including DNA methylation. Moreover, epigenetic regulation of Pomc promoter can affect its expression. MECP2 is a key methyl-CpG-binding protein that is recruited to methylated DNA to regulate gene expression. Our aim is to examine the regulation of hypothalamic Pomc expression by MECP2, and whether and how this contributes to leptin resistance.

**Materials and methods:** A mouse line with MECP2 specifically deleted in POMC neurons was generated and metabolic phenotypes were analyzed in the male mice. Hypothalamic Pomc expression and Pomc promoter methylation was investigated to understand the underlying mechanism.

**Results:** The knockout (KO) mice were normal at birth, but started to show significantly higher bodyweight at the age of 15 weeks, with significantly higher body fat and lower body lean percentage. Metabolic chamber analysis revealed that the KO mice had increased food intake, higher respiratory exchange ratio (RER), and higher energy expenditure. Furthermore, the KO mice showed elevated leptin levels, suggesting the mice have developed leptin resistance. Pomc mRNA was found to be significantly downregulated in the hypothalamus of the KO mice. Finally, DNA methylation on Pomc promoter in the hypothalamus was significantly higher in the KO mice.

**Conclusion:** Our results show that lack of MECP2 in POMC neurons is associated with increased DNA methylation on Pomc promoter and decreased Pomc expression in the hypothalamus, which may account for the observed leptin-resistant phenotypes, such as increased food intake and higher body weight. This study demonstrates that MECP2 is a positive regulator of Pomc expression in the hypothalamus, and DNA methylation on Pomc promoter affects Pomc expression in the hypothalamus and contributes to leptin resistance.

Supported by: A\*STAR BMRC



## OP 06 What's new in the treatment of diabetic nephropathy?

31

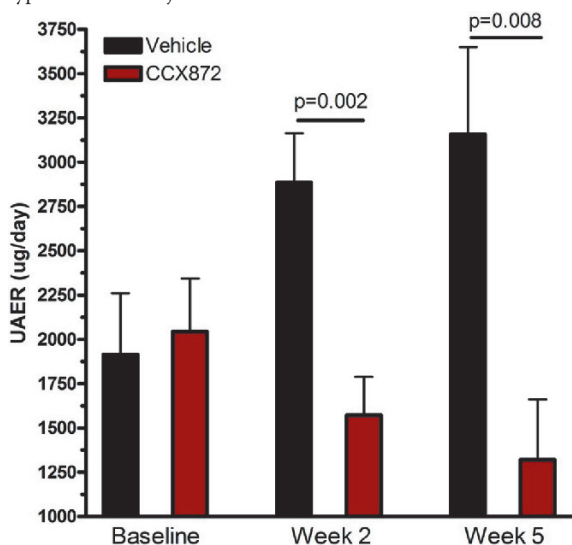
**CCR2 inhibition improves renal function in diabetic BTBR ob/ob Mice**  
T.J. Sullivan, Z. Miao, R. Berahovich, B.N. Zhao, L. Ertl, T. Baumgart, A. Krasinski, T. Dang, S. Miao, J.P. Powers, J.C. Jaen, T.J. Schall; Biology, ChemoCentryx, Inc., Mountain View, USA.

**Background and aims:** Diabetic nephropathy represents a major complication of uncontrolled diabetes. The chemokine receptor CCR2 has been implicated in the recruitment of blood monocytes into kidney in response to stressors such as hypertension and hyperglycemia. In addition, parenchymal renal cells are thought to upregulate CCR2 under those pathological conditions. We describe for the first time the renal benefits associated with CCR2 inhibition in diabetic BTBR *ob/ob* mice, which develop a constellation of abnormalities that closely resemble advanced human diabetic nephropathy and has been proposed as one of the best mouse models for evaluating the efficacy of diabetic nephropathy therapeutics.

**Materials and methods:** CCX872 is a potent and selective, orally bioavailable small molecule CCR2 antagonist, which has previously shown efficacy on hyperglycemia and impaired renal function in various preclinical models (e.g., BKS *db/db* mice). In the present study, CCX872 was dosed daily to male BTBR *ob/ob* mice (age 8 or 16 weeks) for up to 12 weeks. Doses of CCX872 were selected to provide high (>90%) inhibition of blood leukocyte CCR2 throughout the course of the day. Weekly assessments included body weights, fasting plasma glucose, serum clinical chemistry panel, and 24-hr urinary volume and output of albumin, creatinine and glucose.

**Results:** Treatment with CCX872 for 5 weeks significantly reduced urinary albumin excretion rate (UAER) (CCX872: 1322 µg/day vs. vehicle control: 3158 µg/day;  $p=0.008$ ) and albumin:creatinine ratio (ACR) (CCX872: 3559 µg/mg vs. vehicle control: 6740 µg/mg;  $p=0.02$ ). Statistically significant improvements in UAER (1572 µg/day vs. 2882 µg/day;  $p=0.002$ ) and urinary ACR (4418 µg/mg vs. 9118 µg/mg;  $p=0.005$ ) were seen as early as 2 weeks after initiation of CCX872 treatment. Serum markers of renal function were also improved after 5 weeks of CCX872 treatment: serum creatinine (0.32 mg/dL vs. 0.67 mg/dL;  $p=0.04$ ) and blood urea nitrogen (32 mg/dL vs. 100 mg/dL;  $p=0.05$ ). The benefits seen on renal function coincided with significantly reduced glomerular hyperfiltration, as determined by inulin-FITC clearance (679 µl/min vs. 1108 µl/min;  $p=0.03$ ). CCX872 was well tolerated and had no effects on body weights throughout the study.

**Conclusion:** Robust and rapid improvements of albuminuria, serum creatinine and blood urea nitrogen, and normalization of glomerular hyperfiltration were seen following administration of CCX872 to BTBR *ob/ob* mice suffering from pronounced hyperglycemia and impaired renal function. These results support the clinical evaluation of CCR2 antagonists for the treatment of diabetic nephropathy. Our group is currently conducting two Phase 2 diabetic nephropathy trials with CCX140-B, another orally active CCR2 antagonist that recently showed promising anti-diabetic effects in a Phase 2 trial in type 2 diabetic subjects.



32

**Association between glycation gap and macroproteinuria in type 2 diabetic patients**

I. Banu, E. Cosson, Y. Jaber, S. Chiheb, C. Cussac-Pillegand, M. Nguyen, N. Charnaux, P. Valensi; Dept of Endocrinology-Diabetology-Nutrition, Jean Verdier Hospital, AP-HP, CRNH-IdF, Paris-Nord University, Bondy, France.

**Background and aims:** Variation among diabetic patients in the intracellular glycation of proteins, independent of glycemia, could contribute to diabetic complications. The aim of the study was to evaluate a potential association between glycation gap (GG), i.e. the difference of glycation in the intra-erythrocyte compartment (HbA1c) and in the extracellular compartment (fructosamine), and complications in type 2 diabetic patients.

**Materials and methods:** We included 486 type 2 diabetic patients with high cardiovascular risk who underwent cardiovascular examinations including the detection of silent myocardial ischemia by stress scintigraphy and had concomitant measurement of HbA1c (turbimetric immuno-assay) and fructosamine (colorimetry). We assessed predicted HbA1c (HbA1c-F) from the correlation between fructosamine and HbA1c (HbA1c-F =  $(0.021 \times \text{fructosamine}) + 2.025$ ;  $r=0.72$ ;  $p<0.001$ ) and created 3 groups: "highGG" (the 5% patients with the highest HbA1c as compared to HbA1c-F), "lowGG" (5% in mirror) and "normalGG" (90% intermediate). A second series of 459 type 2 diabetic patients with a lower cardiovascular risk and no cardiac exploration was also tested.

**Results:** Body mass index (highGG / normalGG / lowGG  $28 \pm 7 / 30 \pm 6 / 32 \pm 8$  kg/m<sup>2</sup>,  $p=0.03$ ), HbA1c ( $7.0 \pm 1.5 / 8.7 \pm 2.0 / 12.3 \pm 1.5\%$ ,  $p<0.01$ ) and the prevalence of macroproteinuria ( $4.2 / 10.6 / 32.0\%$ ,  $p<0.01$ ) differed across GG groups, without significant difference for retinopathy, peripheral neuropathy nor silent myocardial ischemia. The association of GG groups with macroproteinuria persisted after adjustment on gender, age, body mass index and HbA1c (estimate -1.53,  $p=0.005$ ). Patients with vs without macroproteinuria had GG  $0.562 \pm 1.603$  vs  $-0.058 \pm 1.426$ ;  $p<0.01$ , and this difference persisted after adjustment. The results were concordant in the second series: macroproteinuria according to GG groups  $0 / 4.1 / 14.3\%$ ,  $p<0.01$  after adjustment; higher GG in macroproteinuric patients ( $0.481 \pm 1.105$  vs  $0.027 \pm 1.105$ ,  $p<0.05$  after adjustment).

**Conclusion:** In type 2 diabetic patients, GG is associated with macroproteinuria, independently of HbA1c and other confounding factors, suggesting a specific role of glycation susceptibility on kidney glomerule.

33

**Liver X receptor agonist ameliorates diabetic nephropathy by inhibiting high glucose-induced osteopontin expression in proximal tubular epithelial cells**

D. Ogawa;

Department of Diabetic Nephropathy, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan.

**Background and aims:** Osteopontin (OPN) is a proinflammatory cytokine implicated in the chemoattraction of monocytes and the development of diabetic nephropathy. Synthetic agonists for the Liver X Receptor (LXR), prevent the development of atherosclerosis by regulating cholesterol homeostasis and suppressing inflammatory gene expression, however, the role of LXR in diabetic nephropathy is poorly understood.

**Materials and methods:** We administered LXR agonist T0901317 (10 mg/kg/day) to STZ induced-diabetic mice for 8 weeks after inducing diabetes, and evaluated the effects for diabetic nephropathy. The mechanism of OPN expression was also analyzed in mProx24 cells, a mouse renal proximal tubular epithelial cell line, stimulated with high glucose medium and pretreated with T0901317.

**Results:** T0901317 decreased urinary albumin excretion without altering blood glucose levels. Macrophage infiltration, mesangial matrix accumulation, and interstitial fibrosis were substantially attenuated by T0901317. The gene expression of inflammatory mediators including OPN in the kidney cortex was also suppressed. *In vitro* studies demonstrated that LXR activation suppressed the expression of OPN in proximal tubular epithelial cells. This inhibition was mediated through an inhibition of AP-1 dependent transcriptional activation of the OPN promoter.

**Conclusion:** These findings uncover a previously unrecognized mechanism for the inhibition of renal OPN expression by activation of LXR and support the concept that LXR agonists may offer a novel therapeutic approach for the treatment of diabetic nephropathy.

## 34

**Metformin protects kidney cells from methylglyoxal-mediated damage**T.L. Buxton<sup>1</sup>, T. Loganathan<sup>1</sup>, J. Mabley<sup>2</sup>;<sup>1</sup>Brighton and Sussex Medical School, <sup>2</sup>School of Pharmacy & Biomolecular Sciences, University of Brighton, UK.

**Background and aims:** Methylglyoxal (MGO), a glycolysis derived reactive dicarbonyl compound has been implicated as a central mediator of diabetic complications in both Type I and Type II diabetes. Diabetes-mediated kidney complications are a leading cause of morbidity and mortality in this patient group and understanding the pathological mechanisms involved will allow for development of effective therapies. The aim of this study is twofold, firstly to examine the effects of MGO on kidney cell viability and secondly as metformin has a similar structure to aminoguanidine, a known scavenger of MGO, to investigate whether metformin could provide any direct protective effect against MGO-mediated deleterious effects

**Materials and methods:** The rat proximal tubular cell line, NRK-52E, was exposed to MGO (0.1–1 mM) for 24 h. Cell viability was determined using the MTT assay and cell death assessed by morphological analysis following propidium iodide/Hoechst staining. Oxidative stress was measured using the nitroblue tetrazolium assay. NRK-52E cells were also exposed to MGO (0.6–1 mM) ± aminoguanidine (0.1–0.5 mM) or metformin (0.1–0.5 mM) and again cell viability, apoptosis and necrosis levels, and oxidative stress was measured.

**Results:** NRK-52E cells exposed to MGO for 24 h showed a dose-dependent decrease in cell viability with 0.6 mM reducing it to 41 ± 1% ( $p < 0.05$  vs. untreated cells). There was also an increase in both necrosis, from 3 ± 1% to 41 ± 5%, and apoptosis, from 3 ± 1% to 14 ± 3%, following 0.6 mM MGO exposure ( $p < 0.05$  vs. untreated cells). MGO 0.6 mM also increased cellular oxidative stress following a 6 h exposure by 37 ± 7% ( $p < 0.05$  vs. untreated cells). Aminoguanidine proved effective in providing significant protection against MGO-mediated damage including at MGO concentrations of 0.8 and 1 mM. Metformin provided significant protection but was less effective than aminoguanidine providing protection only against MGO concentrations of 0.6 mM or lower. Metformin, 0.3 and 0.5 mM, increased cell viability to 54 ± 2% and 63 ± 3% respectively ( $p < 0.05$  vs. 0.6 mM MGO alone). Similar protective effects of metformin, 0.3 and 0.5 mM, against MGO-mediated increases in necrosis and apoptosis were also observed, necrosis being reduced to 17 ± 4% and 7 ± 3% ( $p < 0.05$  vs. 0.6 mM MGO) and 5 ± 2% ( $p < 0.05$  vs. 0.6 mM MGO alone) respectively. Both aminoguanidine and metformin reduced the MGO-mediated increase in cellular oxidative stress.

**Conclusion:** Physiological concentrations of MGO reduced rat kidney proximal tubular cell viability and increased levels of both necrosis and apoptosis. These effects may be mediated by the increased cellular oxidative stress observed following MGO exposure. Metformin was able to provide significant protection against the MGO-mediated loss of proximal tubule cell viability and increased apoptosis and necrosis. This data suggests that metformin, apart from its glucose-lowering effect, may also provide direct protection against methylglyoxal-mediated diabetic complications.

## 35

**DPP-4 inhibition with linagliptin delays the progression of diabetic nephropathy in db/db mice**Y. Sharkovska<sup>1</sup>, M. Alter<sup>2,3</sup>, C. Reichetzer<sup>2,4</sup>, O. Tsuprykov<sup>4</sup>, T. Klein<sup>5</sup>, B. Hoher<sup>4</sup>;<sup>1</sup>Institute of Vegetative Anatomy Charité Universitätsmedizin, Berlin,<sup>2</sup>Center for Cardiovascular Research / Institute of Pharmacology, Berlin,<sup>3</sup>Department of Nephrology, Charité Campus Benjamin Franklin, Berlin,<sup>4</sup>University of Potsdam, <sup>5</sup>Boehringer Ingelheim, Biberach, Germany.

**Background and aims:** Diabetic nephropathy is the main cause of end-stage renal disease worldwide. This study aimed to determine the effect of the dipeptidyl peptidase (DPP)-4 inhibitor linagliptin on diabetic nephropathy, independently of its glucose-lowering action.

**Materials and methods:** Male diabetic *db/db* mice at 10 weeks were divided into 3 groups and followed for a total of 12 weeks: vehicle ( $n = 10$ ), linagliptin 3 mg/kg/day ( $n = 8$ ) and enalapril 20 mg/kg/day ( $n = 10$ ). Heterozygous mice treated with vehicle were used as controls ( $n = 8$ ). Serum and urine samples were analysed for glucose, triglycerides, insulin, cystatin C and creatinine at baseline and monthly thereafter. Body weight, urinary albumin excretion and OGTT were monitored periodically. Renal histology (glomerulosclerosis, tubulointerstitial fibrosis) and expression of podocalyxin, glucagon-like pep-

tide-1 receptor (GLP-1R),  $\alpha$ -smooth muscle actin and type I collagen were evaluated at the end of the study.

**Results:** Compared with healthy control *db/+* mice, diabetic *db/db* mice showed substantially higher body weight and fasting serum levels of glucose, insulin and triglycerides. Neither linagliptin nor enalapril had significant effects on glucose levels and did not modify glucose disposal following OGTT in diabetic *db/db* mice. Urinary albumin excretion rates and tubulointerstitial fibrosis were significantly decreased in *db/db* mice treated with linagliptin compared with those treated with enalapril (both  $P < 0.05$ ). Glomerular mesangial matrix expansion in both treatment groups was reduced to almost the level observed in healthy control mice ( $P < 0.05$ ). The expression of podocalyxin in the glomeruli of *db/db* mice was lower than in healthy control mice ( $1.59 \pm 0.2$  vs.  $2.65 \pm 0.1$ ;  $P < 0.001$ ). Podocalyxin expression increased in the glomeruli of mice treated with linagliptin ( $2.31 \pm 0.15$ ) and enalapril ( $2.42 \pm 0.17$ ) compared with vehicle-treated diabetic *db/db* mice (both  $P < 0.05$ ). The expression pattern of  $\alpha$ -smooth muscle actin was also determined in kidneys as a marker of mesangial cell damage. Linagliptin treatment normalized the expression of  $\alpha$ -smooth muscle actin-positive myofibroblasts in the interstitium and glomeruli of diabetic *db/db* mice. Similar results were obtained for type I collagen deposition. Immunohistochemical staining of kidney sections revealed a decrease in GLP-1R expression in the cortical glomeruli of *db/db* mice ( $1.67 \pm 0.07$ ) compared with healthy control mice ( $2.15 \pm 0.1$ ;  $P < 0.01$ ). Linagliptin treatment significantly increased the expression of GLP-1R in the glomeruli of *db/db* mice ( $1.90 \pm 0.04$ ;  $P < 0.05$ ) compared with vehicle-treated diabetic *db/db* mice.

**Conclusion:** This study suggests that treatment with the DPP-4 inhibitor linagliptin protects against the progression of diabetic nephropathy independently of its effect on glucose lowering. The renoprotective action of linagliptin may be due to the inhibition of podocyte damage and myofibroblast transformation.

Supported by: *Boehringer Ingelheim*

## 36

**Effects of the DPP-4 inhibitor linagliptin on albuminuria in patients with type 2 diabetes and diabetic nephropathy**P.-H. Groop<sup>1</sup>, M. Cooper<sup>2</sup>, V. Perkovic<sup>3</sup>, A. Emser<sup>4</sup>, T. Seck<sup>4</sup>, M. von Eynatten<sup>4</sup>, H.-J. Woerle<sup>4</sup>;<sup>1</sup>Department of Medicine, Division of Nephrology, Helsinki UniversityCentral Hospital, Finland, <sup>2</sup>The Baker IDI Heart and Diabetes Institute,Melbourne, Australia, <sup>3</sup>The George Institute for Global Health, Sydney,Australia, <sup>4</sup>Boehringer Ingelheim, Ingelheim, Germany.

**Background and aims:** Diabetes mellitus has become the most common single cause of end-stage renal disease and a high proportion of individuals with type 2 diabetes (T2D) are found to have microalbuminuria and overt nephropathy shortly after the diagnosis of their diabetes. Linagliptin, a DPP-4 inhibitor, has recently demonstrated glycaemic efficacy and safety in T2D patients at advanced stages of kidney disease. Here we report the clinical effect of linagliptin on albuminuria in T2D patients with early diabetic nephropathy.

**Materials and methods:** Seven randomised, double-blind, placebo-controlled trials (duration 24–52 weeks) of linagliptin as monotherapy or add-on to various glucose-lowering background therapies had data available for urinary albumin-to-creatinine ratio (UACR) and were eligible for this analysis ( $n = 4113$ ). Data after 24 weeks of treatment were generated to allow pooling and two sets were defined: 1) Diabetic nephropathy in earlier stages of T2D (with and without oral glucose-lowering background therapies): participants from four 24-week pivotal Phase 3 trials if they had persistent albuminuria, defined as  $30 \leq \text{UACR} \leq 3000$  mg/g and stable treatment with an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB) at baseline; 2) Diabetic nephropathy in elderly patients (various glucose-lowering background therapies including insulin): patients from all seven trials, fulfilling UACR criteria and aged  $\geq 65$  years. The endpoint in both sets was the percentage change in geometric mean UACR after 24 weeks.

**Results:** For set #1, 492 out of 2472 patients met UACR criteria of whom 46% received stable ACEi/ARB therapy (linagliptin,  $n = 168$ ; placebo,  $n = 59$ ). For set #2, 1331 patients were aged  $\geq 65$  years of whom 377 (28%) met UACR criteria (linagliptin,  $n = 232$ ; placebo,  $n = 145$ ). Mean baseline HbA1c and median UACR were 8.3% and 76 mg/g overall for set #1, and 8.1% (overall), 77 mg/g (linagliptin) and 86 mg/g (placebo) for set #2. In set #1, placebo-corrected changes in HbA1c and fasting plasma glucose were -0.71% and -1.4 mmol/L (-26 mg/dL), respectively (both  $P < 0.0001$ ). Linagliptin significantly lowered adjusted UACR by 33% (95% CI: 22%, 42%;  $P < 0.05$ ) with a between-group



difference versus placebo of -29% (95% CI: -3%, -48%;  $P<0.05$ ). In set #2, linagliptin also significantly lowered adjusted UACR by 30% (95% CI: 13%, 43%;  $P<0.05$ ) with a trend towards a reduction versus placebo of -25% (95% CI: -47%, +6%). In all seven studies, blood pressure and renal function were not affected to a clinically meaningful extent by either treatment.

**Conclusion:** In studies up to 52 weeks, linagliptin lowered albuminuria beyond what may be expected by its glucose-lowering effects. Potential long-term renal benefit of linagliptin needs to be explored.

*Supported by: Boehringer Ingelheim*

## OP 07 What's new in insulin therapy?

### 37

#### Effect of peripheral delivery of a liver preferential insulin analogue on glucose and fat metabolism

D.S. Edgerton<sup>1</sup>, M. Scott<sup>1</sup>, J. Roop<sup>1</sup>, D. Neal<sup>1</sup>, P. Williams<sup>1</sup>, T.B. Kjeldsen<sup>2</sup>, P. Madsen<sup>2</sup>, H. Naver<sup>2</sup>, C.B. Jeppesen<sup>2</sup>, E. Nishimura<sup>2</sup>, C.L. Brand<sup>2</sup>, A.D. Cherrington<sup>1</sup>;

<sup>1</sup>Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, USA, <sup>2</sup>Novo Nordisk, Copenhagen, Denmark.

**Background and aims:** Endogenous insulin secretion exposes the liver to concentrations of insulin which are ~2.5-fold greater than at non-hepatic tissues. Subcutaneous insulin delivery eliminates this gradient and is associated with metabolic abnormalities. Thus, restoring the physiologic gradient may provide a therapeutic benefit. The hepato-selectivity of a novel insulin analog (insulin 327) was tested.

**Materials and methods:** Dogs with arterial, portal vein and hepatic vein catheters were studied after an 18h fast. 3H-glucose was infused from -140 min. After a basal period (-40 to 0 min) somatostatin and basal portal glucagon were infused (0 to 300 min). At the same time insulin 327 or human insulin (HI) was infused into a peripheral vein (7.2 or 1.8 pmol/kg/min, respectively;  $n=5$ /group) and euglycemia was maintained by glucose infusion.

**Results:** Arterial insulin 327 and HI levels increased to  $10870 \pm 2543$  and  $95 \pm 8$  pmol/l, respectively. Although the glucose infusion rates were similar in insulin 327 and HI ( $3.1 \pm 0.3$  vs  $3.1 \pm 1.6$  mg/kg/min, respectively, last 3h), insulin 327 had a greater effect on the liver and a lesser effect on non-hepatic tissues compared to HI. Relative to the basal period, insulin 327 suppressed net hepatic glucose balance and glucose Ra (mg/kg/min; last 3h) by  $1.9 \pm 0.1$  and  $1.7 \pm 0.1$ , respectively, while HI reduced these by only  $0.5 \pm 0.6$  and  $1.1 \pm 0.3$ . On the other hand, insulin 327 only increased non-hepatic glucose uptake and glucose Rd (mg/kg/min; last 3h) by  $1.0 \pm 0.4$  and  $1.5 \pm 0.3$ , respectively, while HI increased these by  $2.4 \pm 1.2$  and  $2.5 \pm 1.1$ . Suppression of lipolysis was delayed with insulin 327 compared to HI: during the 1st hour plasma NEFA levels ( $\mu\text{mol/l}$ ) decreased by  $91 \pm 37$  vs  $419 \pm 42$ , respectively, and blood glycerol levels ( $\mu\text{mol/l}$ ) fell by  $0 \pm 7$  vs  $39 \pm 5$ , respectively. During the last 3h, however, there were no differences in the suppression of lipolysis by insulin 327 compared to HI.

**Conclusion:** These results demonstrate the efficacy of a peripherally delivered insulin analog designed to preferentially target liver glucose metabolism.

*Supported by: Novo Nordisk*

### 38

#### Two phase 3 trials of 3-times weekly insulin degludec versus once-daily insulin glargine in insulin-naïve people with type 2 diabetes

J.H. DeVries<sup>1</sup>, R.E. Ratner<sup>2</sup>, B.W. Bode<sup>3</sup>, D.L. Russell-Jones<sup>4</sup>, K. Begtrup<sup>5</sup>, T. Johansen<sup>5</sup>, B. Zinman<sup>6</sup>;

<sup>1</sup>Academic Medical Center, Amsterdam, Netherlands, <sup>2</sup>MedStar Health Research Institute, Hyattsville, USA, <sup>3</sup>Atlanta Diabetes Associates, Atlanta, USA, <sup>4</sup>Royal Surrey County Hospital, Guildford, UK, <sup>5</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>6</sup>Samuel Lunenfeld Research Institute, Toronto, Canada.

**Background and aims:** Insulin degludec (IDeg) is an ultra-long-acting basal insulin with a half-life of ~25 h (twice that of insulin glargine (IGlar)) and a consistent action profile of >42 h. Based on these characteristics, a 3-times weekly dosing regimen (3TW) was explored in a treat-to-target phase 2 trial in patients with type 2 diabetes (T2D) where IDeg 3TW was as safe and efficacious as once-daily IGlar (IGlar OD). Here we present results of two phase 3 trials designed to further evaluate efficacy and safety of IDeg 3TW.

**Materials and methods:** In two 26-week, open-label, non-inferiority, treat-to-target trials in insulin-naïve subjects with T2D, IDeg was injected Mon, Wed & Fri before the first meal of the day (3TW AM: Trial 1) or from evening meal to bedtime (3TW PM: Trial 2), and compared with IGlar OD (dosed the same time each day as per label). In total, 927 subjects (mean: age  $57.7 \pm 10$  yrs,  $\text{HbA}_{1c}$   $8.3 \pm 0.8\%$ , diabetes duration  $8.8 \pm 6.1$  yrs, BMI  $32.3 \pm 5.3$  kg/m<sup>2</sup>) were randomised (1:1) to IDeg 3TW or IGlar OD, combined with metformin +/- DPP-4 inhibitors. Starting doses were 10 U/day (IGlar OD) or 20 U/injection (IDeg 3TW); doses were titrated to FPG <5 mmol/L using a common algorithm (IDeg 3TW dose adjustments were twice those of IGlar OD).

**Results:** IDeg 3TW and IGlar OD decreased  $\text{HbA}_{1c}$  from baseline by 1.0-1.1% and 1.4%, respectively. However, non-inferiority was not confirmed

(3TW AM-IGlar OD: 0.34% [0.18; 0.51]95%CI; 3TW PM-IGlar OD: 0.26% [0.11; 0.41]); thus, the primary outcome was not achieved. For IDeg 3TW, pre-breakfast SMPG levels gradually increased with time since last injection (Table). At Week 26, calculated mean insulin utilization was 50–51 U/day (IDeg 3TW) and 56–62 U/day (IGlar OD). Overall rates of confirmed hypoglycaemia (PG <3.1 mmol/L or requiring assistance) were 1.0–1.6 episodes per patient-yr of exposure (PYE) and similar for 3TW AM and IGlar OD (estimated rate ratio: 1.04 [0.69; 1.55]), but higher for 3TW PM vs. IGlar OD (1.58 [1.03; 2.43]). Overall rates of nocturnal confirmed hypoglycaemia were 0.2–0.4 episodes per PYE, and similar for 3TW PM and IGlar OD (0.60 [0.21; 1.69]), but higher for 3TW AM vs. IGlar OD (2.12 [1.08; 4.16]). As non-inferiority of HbA<sub>1c</sub> was not obtained, hypoglycaemia results should be interpreted with caution as the difference at similar HbA<sub>1c</sub> may be even greater. In total, 2 severe hypoglycaemic episodes were reported for IDeg 3TW (1 with AM dosing; 1 with PM dosing) vs. 1 episode with IGlar OD.

**Conclusion:** While the clinically relevant reductions in HbA<sub>1c</sub> confirm the ultra-long duration of action of IDeg, the overall benefit to risk relationship in these phase 3 trials does not support a 3TW dosing regimen in this patient population. Thus, IDeg should only be recommended for once-daily use.

	Mean pre-breakfast self-measured PG (mmol/L) - Week 26			
	IDeg 3TW AM	IGlar OD*	IDeg 3TW PM	IGlar OD*
<b>One day since 3TW injection</b> (Tue, Thur, Sat)	5.98	6.07	5.85	5.83
<b>Two days since 3TW injection</b> (Wed, Fri, Sun)	6.91	6.08	6.49	5.84
<b>Three days since 3TW injection</b> (Mon)	7.50	6.05	7.04	5.91

\*IGlar was dosed at the same time each day according to label

Clinical Trial Registration Number: NCT01076647 / NCT01068678

Supported by: Novo Nordisk

## 39

### Effect of insulin degludec on glycaemic control and nocturnal hypoglycaemia compared with insulin glargine: a 1-year trial in insulin-naïve patients with type 2 diabetes

**B. Zinman**<sup>1</sup>, A. Philis-Tsimikas<sup>2</sup>, Y. Handelsman<sup>3</sup>, H.W. Rodbard<sup>4</sup>, B. Cariou<sup>5</sup>, T. Johansen<sup>6</sup>, L. Endahl<sup>6</sup>, C. Mathieu<sup>7</sup>

<sup>1</sup>Samuel Lunenfeld Research Institute, Toronto, Canada, <sup>2</sup>Scripps Whittier Diabetes Institute, La Jolla, USA, <sup>3</sup>Metabolic Institute of America, Tarzana, USA, <sup>4</sup>Endocrine and Metabolic Consultants, Rockville, USA, <sup>5</sup>Department of Endocrinology, Nantes University Hospital, Nantes, France, <sup>6</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>7</sup>UZ Gasthuisberg, KU Leuven, Belgium.

**Background and aims:** Insulin degludec (IDeg) is a novel basal insulin with an ultra-long, flat action profile. The objective of this 52-week randomised, open-label, non-inferiority, treat-to-target trial was to compare the efficacy and safety of IDeg to insulin glargine (IGlar) given s.c. once daily in insulin-naïve patients with type 2 diabetes inadequately controlled with oral antidiabetic drugs (metformin±dipeptidyl peptidase-4 inhibitor).

**Materials and methods:** 1030 adults (mean age 59.1 years; diabetes duration 9.2 years; baseline HbA<sub>1c</sub> 8.2%; fasting plasma glucose [FPG] 9.7 mmol/L) were randomised 3:1 to IDeg or IGlar. Both basal insulins were titrated to achieve self-measured blood glucose targets calibrated to PG of 3.9–4.9 mmol/L.

**Results:** Patient completion rates were 79% (IDeg) and 77% (IGlar). At 1 year, IDeg reduced HbA<sub>1c</sub> (−1.06%) non-inferior to IGlar (−1.19%) (estimated treatment difference [ETD] IDeg-IGlar: 0.09% [95% CI: −0.04; 0.22]). FPG reductions were significantly larger with IDeg than IGlar (−3.76 vs. −3.30 mmol/L; ETD: −0.43 mmol/L [95%CI: −0.74; −0.13];  $p=0.005$ ). Overall confirmed hypoglycaemia (PG <3.1 mmol/L and severe episodes requiring assistance) rates were similar for IDeg and IGlar (1.52 vs. 1.85 episodes/patient-year; estimated rate ratio [ERR] IDeg:IGlar 0.82 [95% CI: 0.64; 1.04];  $p=0.11$ ). Overall severe hypoglycaemia was infrequent but significantly lower with IDeg (0.003 vs. 0.023 episodes/patient-year; ERR IDeg:IGlar 0.14 [95% CI: 0.03; 0.70];  $p=0.02$ ). Nocturnal confirmed hypoglycaemia rates were significantly 36% lower with IDeg (0.25 vs. 0.39 episodes/patient-year; ERR IDeg:IGlar 0.64 [95% CI: 0.42; 0.98];  $p=0.04$ ). End-of-trial mean daily insulin

doses were 0.59 (IDeg) and 0.60 (IGlar) U/kg. Mean weight gain was similar: 2.4 kg for IDeg and 2.1 kg for IGlar. Overall adverse event rates were low and similar between groups.

**Conclusion:** In this treat-to-target trial with insulin-naïve patients with type 2 diabetes, IDeg and IGlar provided similar long-term glycaemic control, with significantly lower rates of nocturnal hypoglycaemia with IDeg.

Clinical Trial Registration Number: NCT00982644

Supported by: Novo Nordisk

## 40

### Insulin degludec is superior to sitagliptin in improving glycaemic control in uncontrolled patients with type 2 diabetes on oral agents

**A. Philis-Tsimikas**<sup>1</sup>, S. Del Prato<sup>2</sup>, I. Satman<sup>3</sup>, A. Bhargava<sup>4</sup>, M. Dharmalingam<sup>5</sup>, T.V. Skjøth<sup>6</sup>, S. Rasmussen<sup>6</sup>, A.J. Garber<sup>7</sup>

<sup>1</sup>Scripps Whittier Diabetes Institute, La Jolla, USA, <sup>2</sup>Department of Endocrinology and Metabolism, University of Pisa, Italy, <sup>3</sup>Istanbul University, Istanbul Faculty of Medicine, Turkey, <sup>4</sup>Iowa Diabetes & Endocrinology Research Center, Des Moines, USA, <sup>5</sup>Bangalore Endocrinology & Diabetes Research Center, India, <sup>6</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>7</sup>Department of Medicine, Baylor College of Medicine, Houston, USA.

**Background and aims:** Insulin degludec (IDeg) is an ultra-long-acting basal insulin with a stable glucose-lowering action profile. The aim of this study was to compare IDeg to sitagliptin (Sita), a dipeptidyl peptidase-4 inhibitor, in insulin-naïve patients with type 2 diabetes who were inadequately controlled with oral antidiabetic drugs (OADs).

**Materials and methods:** In this 26-week, open-label trial 458 insulin-naïve adults with type 2 diabetes (mean age 56 years, diabetes duration 7.7 years, baseline HbA<sub>1c</sub> 8.9% [73.8 mmol/L], fasting plasma glucose [FPG] 9.7 mmol/L [175 mg/dL]) were randomised (1:1) to once-daily IDeg (n=229) or Sita (100 mg orally; n=229) as add-on to stable treatment with one or two OADs (metformin, sulphonylureas, glinides or pioglitazone). IDeg was dosed between wake-up and bedtime based on each individual's preference.

**Results:** The completion rate was 76% in both treatment arms. At 26 weeks, IDeg was superior to Sita in reducing HbA<sub>1c</sub>; (estimated treatment difference [ETD] IDeg-Sita: −0.43% points, [95% CI: −0.61, −0.24;  $p<0.0001$ ] with observed mean reductions of 1.56% vs. 1.22%-points, respectively). HbA<sub>1c</sub> <7% (53 mmol/mol) was achieved by 41% (IDeg) versus 28% (Sita) of patients, estimated odds ratio IDeg:Sita 1.60 [1.04, 2.47;  $p=0.034$ ]. IDeg was superior to Sita in reducing FPG (ETD IDeg-Sita: −2.17 mmol/L [−2.59, −1.74;  $p<0.0001$ ] with observed mean reductions of 3.22 versus 1.39 mmol/L (58 vs. 25 mg/dL), respectively. Despite the lower FPG, there was no statistically significant difference in the rate of nocturnal confirmed hypoglycaemia (PG <3.1 mmol/L [56 mg/dL] or ADA-defined severe episodes from 00:01 to 05:59) between IDeg and Sita (0.52 vs. 0.30 episodes/patient-year, estimated rate ratio (ERR) IDeg:Sita 1.93 [0.90, 4.10;  $p=0.09$ ]). Rates of overall confirmed hypoglycaemia were higher with IDeg than with Sita (3.1 vs. 1.3 episodes/patient-year, ERR IDeg:Sita 3.81 [2.40, 6.05;  $p<0.0001$ ]); one severe episode was reported with IDeg. IDeg was associated with a larger weight gain than Sita: ETD IDeg-Sita 2.75 kg [1.97, 3.54;  $p<0.0001$ ]. The rate of adverse events was low for both groups.

**Conclusion:** IDeg was superior to Sita in improving glycaemic control; overall hypoglycaemia was higher with IDeg, but there was no difference in severe or nocturnal hypoglycaemia. Initiating IDeg in insulin-naïve patients is an effective and well-tolerated alternative to an additional OAD in patients with type 2 diabetes.

Clinical Trial Registration Number: NCT01046110

Supported by: Novo Nordisk

## 41

### Human hyaluronidase + rapid analogue insulin (RAI) improves postprandial glycaemic control in type 1 diabetes compared to insulin lispro alone

**J.S. Skyler**<sup>1</sup>, S. Garg<sup>2</sup>, I.B. Hirsch<sup>3</sup>, T. Blevins<sup>4</sup>, D.E. Vaughn<sup>5</sup>, D.B. Muchmore<sup>5</sup>

<sup>1</sup>University of Miami, <sup>2</sup>University of Colorado Denver, Aurora, <sup>3</sup>University of Washington, Seattle, <sup>4</sup>Texas Diabetes and Endocrinology, Austin, <sup>5</sup>Halozyyme Therapeutics, San Diego, USA.

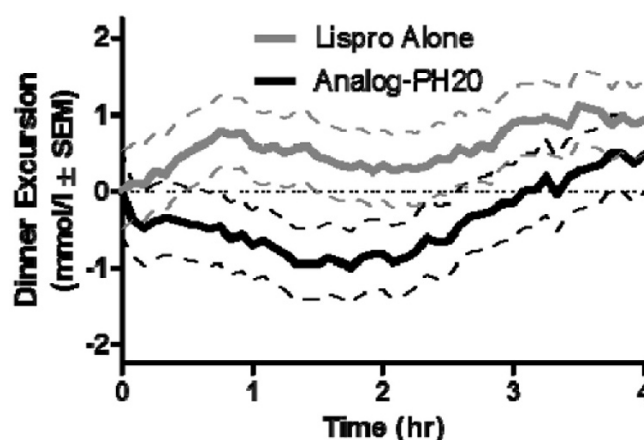
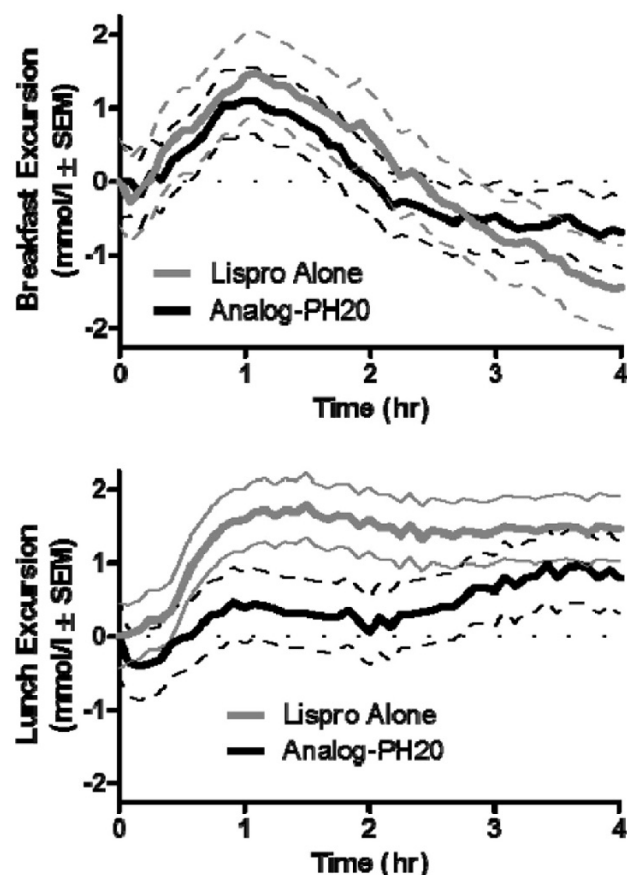
**Background and aims:** Recombinant human hyaluronidase (rHuPH20) accelerates absorption & action of prandial insulins and also reduces postpran-

dial glycemic excursions following liquid test meal administration without increasing risk of hypoglycemia. Our objective was to compare glucose control in T1DM using prandial lispro or RAI+rHuPH20 (Analog-PH20) in the outpatient treatment setting.

**Materials and methods:** After a 4–6 week run-in using prandial glulisine + bid glargine, 117 subjects (age  $43 \pm 14$  years, BMI  $27.3 \pm 4.0$  kg/m<sup>2</sup>, A1C  $7.0 \pm 0.5\%$ ) were randomized (double blind 2-way crossover) to lispro+rHuPH20 or aspart+rHuPH20 vs lispro alone for two 12 week intensive management periods; prandial doses were immediately before meals. The primary endpoint was A1C noninferiority (0.4% margin); postprandial excursions were measured by self monitoring of blood glucose (SMBG) and also by continuous glucose monitoring. Overall hypoglycemia was self-reported as SMBG  $\leq 3.9$  mmol/l or symptoms.

**Results:** Changes from baseline A1C were comparable ( $-0.14\%$  for Analog-PH20;  $-0.19\%$  for lispro), with no significant treatment difference (95% CI  $-0.05, +0.15$ ). At the end of treatment, fasting glucose values were comparable between treatments (Analog-PH20  $7.85 \pm 2.23$  mmol/l vs lispro  $7.90 \pm 2.34$  mmol/l;  $p=.86$ ). Mean postmeal (90 min) excursions were reduced by 82% ( $p=.0045$ ) over the 12 week period with more subjects consistently (at least 2/3 of meals) achieving values  $<10$  mmol/l at breakfast (70.5% vs 54.0%,  $p=.003$ ),  $<10$  mmol/l at all meals (70.8% vs 59.3%,  $p=.016$ ),  $<7.8$  mmol/l at breakfast (21.4% vs. 10.6%,  $p=.007$ ) and  $<7.8$  mmol/l at all meals (15.0% vs 8.8%,  $p=.089$ ). Continuous monitoring over 3 days at the end of treatment showed improved excursion profiles (Figure). The overall hypoglycemia ( $\leq 3.9$  mmol/l or symptoms) rate during the treatment was reduced 5% during Analog-PH20 treatment, from 19.9 to 19.0 episodes per subject-month ( $p=.035$ ) and events  $<3.1$  mmol/l were reduced 7%, from 8.05 to 7.50 per subject-month ( $p=.044$ ). Total daily insulin dose ( $54 \pm 27$  for Analog-PH20 vs  $56 \pm 27$  U for lispro,  $p=.057$ ) and weight gain difference ( $-.26$  kg,  $p=.27$ ) showed favorable trends. Adverse events were comparable between treatments and Analog-PH20 was well tolerated. Immunogenicity results showed 13 subjects with pre-existing anti-rHuPH20 antibodies; 3 subjects developed *de novo*, low titer anti-rHuPH20 antibodies without any associated adverse events.

**Conclusion:** Compared to insulin lispro alone, coformulation of RAI+rHuPH20 improves postprandial glycemic excursions and hypoglycemia risks while maintaining comparable A1C outcomes in an intensive diabetes treatment program.



Clinical Trial Registration Number: NCT00883558

## 42

### LY2605541: Leveraging hydrodynamic size to develop a novel basal insulin

J.M. Beals<sup>1</sup>, G.B. Cutler, Jr.<sup>2</sup>, A. Vick<sup>2,3</sup>, A. Koester<sup>1</sup>, S. Li<sup>1</sup>, A.M. Siesky<sup>1</sup>, R.J. Hansen<sup>1</sup>

<sup>1</sup>Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, <sup>2</sup>formerly of Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, <sup>3</sup>Seventh Wave Laboratories, Chesterfield, USA.

**Background and aims:** Improvements in basal insulins have focused primarily on slowing subcutaneous (SC) release; however, exogenously administered insulin is prone to glomerular filtration and therefore to significant renal clearance. To develop the novel basal insulin analog (LY2605541 [LY]) with the goal of both slowing SC absorption and reducing renal clearance, insulin lispro was site-specifically and covalently modified with a 20-kDa polyethylene glycol (PEG) moiety at lysine B28, via a covalent urethane bond, increasing the hydrodynamic size. LY was also given to 5/6 nephrectomized rats (10 nmol/kg, intravenous injection) to assess the impact of renal impairment on LY clearance.

**Materials and methods:** The hydrodynamic diameter was determined using an ALV/CGS-3 Compact Goniometer system with ALV/LSE-5003 Light Scattering Electronics and Multiple Tau Digital Correlator. LY and insulin lispro were formulated under monomeric conditions with a target concentration of  $\sim 0.5$  mg/mL (to minimize non-ideality) in PBS (pH 7.4) at 298.15K. All sample viscosities were measured at 25°C. Hydrodynamic diameters were derived using the Stokes-Einstein equation. PK/PD studies were conducted in STZ-induced diabetic rats. LY was given as a single subcutaneous injection at doses ranging from 9.4–568 nmol/kg. LY concentrations were determined via immunoassay and glucose levels were determined by glucometer. Allometric scaling was used to project human PK parameters and to simulate once-daily dosing. The 5/6 nephrectomized studies were conducted in Sprague Dawley male rats that underwent a two part surgery (surgeries separated by 1 week; N = 8–10). Blood samples were collected by three methods (retro-orbital bleeds, tail bleeds, or cardiac stick) depending on each test article's time point. Clearance differences were compared to sham controls.

**Results:** Dynamic light scattering analysis indicated that PEGylated insulin lispro (LY) had a hydrodynamic diameter of  $7.9 \pm 0.5$  nm, a diameter 4x larger than insulin lispro and analogous to the size of a  $\sim 75$ kDa globular protein. In rats treated with streptozotocin to induce diabetes, LY's increased hydrodynamic size resulted in a robust dose response profile with slowed absorption from the SC site, exemplified by a shift in  $T_{max}$  ( $\sim 25$ x), and reduced clearance ( $\sim 10$ x) with concomitant glucose-lowering properties. Comparing 5/6th nephrectomized rats with sham controls, no renal clearance differences were observed with LY whereas insulin lispro clearance was reduced. Based upon these preclinical PK/PD data, human plasma profiles were modeled for once-daily dosing of LY. The model predicted gradual attainment of steady-state plasma LY levels with a peak-to-trough ratio of  $<1.5$ .

**Conclusion:** LY2605541 was designed to have a large hydrodynamic size which slows insulin absorption and reduces renal clearance. LY demonstrated prolonged duration of action in preclinical studies. These effects formed the basis for further pursuit of LY as a novel basal insulin, wherein subsequent development confirmed the prolonged PK/PD profile in Phase 1–2 studies.

Supported by: Eli Lilly and Company



## OP 08 Diagnosing and treating diabetic neuropathy

43

### Manifestation of neuropathy in the corneal nerve plexus correlates with hyperglycaemia and increased advanced glycation end products

M. Reichard<sup>1</sup>, H. Weiss<sup>2</sup>, R. Waterstradt<sup>2</sup>, M. Tiedge<sup>2</sup>, O. Stachs<sup>1</sup>, S. Baltrusch<sup>2</sup>,  
<sup>1</sup>Department of Ophthalmology, <sup>2</sup>Institute of Medical Biochemistry and Molecular Biology, University of Rostock, Germany.

**Background and aims:** The most common long-term complication of diabetes mellitus (DM) is the diabetic neuropathy, unfortunately diagnosed at an advanced stage when irreversible damage has already developed. In-vivo confocal laser scanning microscopy (CLSM) facilitates the early examination of alterations in the corneal nerve plexus. However, there is a lack of longitudinal studies dealing with this non-invasive ophthalmic marker in correlation to metabolic parameters in DM. Therefore the aim of this study was to investigate the corneal nerve structure in diabetic and healthy NOD mice by CLSM together with a metabolic characterization of the animals.

**Materials and methods:** The NOD mice were characterised by measuring blood glucose (BG) and glycated HbA<sub>1c</sub>. Advanced glycation end products (AGEs) were determined by ELISA. For CLSM the animals were anesthetized and fixed to avoid movement. The eye was moistened with a carbomer gel and the cornea was visualised using the HRTII + RCM system. Images were quantified for nerve fibre density (NFD) using NeuronJ (ImageJ). Finally the animals were killed and organs were removed for RNA preparation. Expression of the receptor of AGEs (RAGE) was quantified by RT-PCR.

**Results:** Diabetic NOD mice showed at the age of 36 weeks a twofold higher AGEs concentration, and significant alterations in the corneal nerve plexus in comparison to their healthy littermates (n = 10). Mice were monitored over one year with weekly BG measurements. Healthy animals showed mean BG and HbA<sub>1c</sub> concentrations of  $5.6 \pm 0.1$  mmol/l and  $4.1 \pm 0.1$  %, respectively. In healthy animals a NFD of  $19.4 \pm 1.2$  mm/mm<sup>2</sup> was quantified from CLSM image using a semi-automated analysis approach. During diabetes manifestation the BG and HbA<sub>1c</sub> concentration significantly increased ( $17.3 \pm 1.1$  mmol/l and  $11.1 \pm 0.4$  %) whereas inversely a significant decrease in the NFD was observed by CLSM ( $7.0 \pm 0.3$  mm/mm<sup>2</sup>). Diabetic animals (n = 5) were treated with insulin pellets, which were implanted in the subcutaneous fat. The resulting decrease in the BG concentration was reflected in a reduced HbA<sub>1c</sub> concentration ( $7.8 \pm 0.4$  %). The NFD increased in answer to the therapy, but did not recover to the value before diabetes manifestation ( $12.3 \pm 0.2$  mm/mm<sup>2</sup>). Withdrawal of insulin therapy resulted in accelerated NFD loss. In cornea the expression of RAGE was significantly threefold higher than in palaeocortex, cerebellum, cortex, hippocampus, and liver, and further up-regulated in diabetic animals.

**Conclusion:** We could demonstrate for the first time a causal nexus between the increase in BG and AGEs, and the decrease in the corneal nerve plexus. Binding of AGEs to RAGE initiate oxidative stress eventually disable neuronal regeneration and facilitate neuronal dysfunction. The high RAGE expression in the cornea may explain the high susceptibility of the cornea to hyperglycaemia and the early development of neuronal degeneration. Our study indicates that the in-vivo CLSM of the cornea is a valuable early diagnostic criterion of DM.

Supported by: FORUN

44

### Early detection of nerve fibre loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetic subjects

D. Ziegler<sup>1,2</sup>, A. Zhivov<sup>3</sup>, S. Allgeier<sup>4</sup>, K. Winter<sup>5</sup>, N. Papanas<sup>1</sup>, I. Ziegler<sup>1</sup>, J. Brüggemann<sup>1</sup>, S. Peschel<sup>3</sup>, B. Köhler<sup>4</sup>, O. Stachs<sup>3</sup>, R.F. Guthoff<sup>2</sup>, M. Roden<sup>1,2</sup>, for the GDS Group;

<sup>1</sup>Institute for Clinical Diabetology, German Diabetes Center at Heinrich Heine University, Düsseldorf; <sup>2</sup>Department of Metabolic Diseases, University Hospital, Düsseldorf; <sup>3</sup>Department of Ophthalmology, University of Rostock; <sup>4</sup>Institute for Applied Computer Science and Automation, Karlsruhe Institute of Technology; <sup>5</sup>Translational Centre for Regenerative Medicine, University of Leipzig, Germany.

**Background and aims:** Corneal confocal laser scanning microscopy (CCM) has emerged as a noninvasive in vivo technique to quantify the subbasal

nerve plexus (SNP), but it is unclear whether it allows for detection of small nerve fibre pathology at early stages of type 2 diabetes.

**Materials and methods:** We assessed parameters of CCM, corneal sensation, and peripheral nerve function in 86 patients with recently diagnosed type 2 diabetes (71% male, age:  $57.8 \pm 9.1$  [mean $\pm$ SD] years, diabetes duration:  $2.1 \pm 1.8$  years, BMI:  $31.1 \pm 5.7$  kg/m<sup>2</sup>, HbA<sub>1c</sub>:  $6.8 \pm 1.1$ %) and 48 control subjects (65% male, age:  $53.7 \pm 13.4$  years). Intraepidermal nerve fibre density (IENFD) was assessed by immunohistochemistry using PGP 9.5 antibody in 3-mm punch biopsies from the distal leg. Peripheral and autonomic nerve function was quantified by motor and sensory nerve conduction velocity (MNCV, SNCV), sensory nerve action potential (SNAP) amplitudes, vibration perception thresholds (VPT), thermal detection thresholds (TDT), cardiovascular autonomic testing, and pupilligraphy. Image data acquired from automatic CCM focus scans were processed with novel digital image processing algorithms to eliminate motion artifacts and reconstruct images of the SNP devoid of anterior corneal mosaic deformations. Nerve fibres were segmented and analyzed by newly developed software.

**Results:** Corneal nerve fibre density (CNFD) was reduced in patients with diabetes ( $0.0196 \pm 0.007$  vs. control:  $0.0241 \pm 0.007$   $\mu$ m/ $\mu$ m<sup>2</sup>;  $P < 0.001$ ) as were nerve branch density ( $250 \pm 136$  vs.  $327 \pm 171$  mm/m<sup>2</sup>;  $P = 0.008$ ), number of connecting points ( $35.7 \pm 19.0$  vs.  $45.6 \pm 20.0$  mm/m<sup>2</sup>;  $P = 0.005$ ), and nerve fibre thickness ( $2.69 \pm 0.39$  vs.  $2.84 \pm 0.36$   $\mu$ m;  $P = 0.032$ ). CNFD and IENFD were reduced below the 5th percentile in 17% and 18% of the diabetic patients, respectively. However, the vast majority of the patients with abnormal CNFD or IENFD showed concomitantly normal CNFD and abnormal IENFD and vice versa. Either CNFD or IENFD or both parameters were reduced in 34% of the diabetic subjects. In the entire population, CNFD correlated with IENFD ( $r = 0.23$ ;  $P = 0.014$ ), but not with the peripheral, cardiovascular autonomic, and pupillary nerve function tests. In contrast, IENFD correlated significantly with MNCV, SNCV, SNAP amplitudes, TDT, with the closest correlations ( $r > 0.25$ ) being found for sural SNCV and SNAP, median MNCV, and cold TDT on the foot. No difference was noted between patients and controls for corneal sensation.

**Conclusion:** Corneal confocal microscopy detects early nerve fibre loss in recently diagnosed type 2 diabetes, but not necessarily in the same patients as skin biopsy, suggesting a patchy manifestation pattern of small fibre neuropathy in different organs.

45

### Evidence of grey matter atrophy in diabetic neuropathy: a magnetic resonance imaging brain volumetric study

D. Selvarajah<sup>1</sup>, M. Maxwell<sup>1</sup>, J. Davies<sup>1</sup>, A. Sankar<sup>1</sup>, E. Cachia<sup>2</sup>, R. Gandhi<sup>1</sup>, I. Wilkinson<sup>2</sup>, S. Tesfaye<sup>2</sup>;

<sup>1</sup>Diabetes Research Unit, <sup>2</sup>University of Sheffield, UK.

**Background and aims:** Diabetes contributes to accelerated brain atrophy with deleterious effects on cognition leading to dementia. Although the exact mechanisms of brain atrophy remain unknown, chronic metabolic and vascular changes are thought to play an important role. We have previously demonstrated early spinal cord atrophy in subjects with diabetic neuropathy (DN). The aim of this study was to investigate if subjects with DN have evidence of increased brain atrophy.

**Materials and methods:** Forty eight subjects with type 1 diabetes [No-DN (n=22), Painful-DN (n=14), Painless-DN (n=12),] underwent detailed clinical and neurophysiological assessment to quantify the severity of DPN [NIS(LL)+7 tests]. The severity of diabetic retinopathy (DR) was scored from annual eye screening retinal photography. This was used to quantify the severity of microangiopathy. All subjects, including 17 healthy volunteers (HV), underwent volumetric (0.8x0.8x0.8mm<sup>3</sup> resolution) brain magnetic resonance imaging at 3T. Images were analysed using FSL (fMRIB, Oxford, UK); a library of analysis tools for brain imaging data. Peripheral and deep grey matter volumes were calculated using the SIENAX and FIRST tools.

**Results:** All groups were matched for gender. There were significant differences in age [HV 48.6(14.3), No-DN 37.3(10.7), Painless DN 51.4(11.2), Painful-DN 45.8(12.4); ANOVA,  $p = 0.004$ ] and DR score (diabetes groups only;  $p = 0.004$ ) between groups. Group mean differences we examined using a random effects model using age, gender and DR score as covariates (ANCOVA). Painful-DN [760(388) ml] and Painless-DN [767(209) ml] had the lowest total grey matter volume compared to No-DN [812(471) ml] and HV [798(554) ml;  $p = 0.01$ ]. The distribution of brain atrophy was mainly peripheral [HV 631(464) ml, No-DN 640(372) ml, Painful-DN 601(337) ml and Painless DN 605(163) ml;  $p = 0.02$ ] with relative sparing of deep grey matter volume [HV 167(133) ml, No-DN 171(123) ml, Painful-DN 159(101) ml and Painless DN 162(95) ml;  $p = 0.14$ ]. There were no significant differences in white matter

volume ( $p=0.90$ ) and ventricular cerebrospinal fluid volume ( $p=0.40$ ) across the study groups. No significant differences were found between Painful and Painless-DN groups.

**Conclusion:** Our findings suggest the presence of increased peripheral grey matter atrophy in both painful and painless DN. This appears independent of microangiopathy and possibly highlights an important role of the neuro-pathic process in contributing to brain atrophy. This study also provides us with new insights into the extent of central nervous system involvement in DN and may have important implications to its long term prognosis.

*Supported by: Juvenile Diabetes Research Fund*

## 46

### Altered brain microstructure assessed by diffusion tensor imaging in patients with diabetes mellitus and gastrointestinal symptoms

E. Søfteland<sup>1,2</sup>, J.B. Frøkjær<sup>3,4</sup>, L.W. Andersen<sup>3</sup>, G. Dimcevski<sup>1,2</sup>, C. Brock<sup>4</sup>, M. Simrén<sup>5</sup>, A.M. Drewes<sup>4</sup>

<sup>1</sup>Department of Medicine, Haukeland University Hospital, Bergen, Norway,

<sup>2</sup>Institute of Medicine, University of Bergen, Norway, <sup>3</sup>Department of

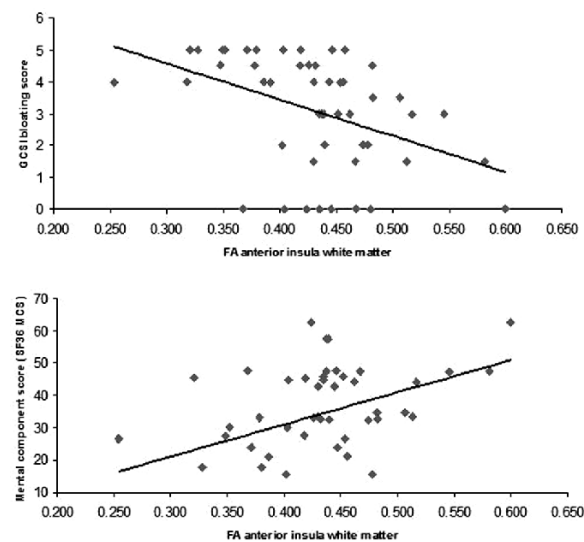
Radiology, Aalborg Hospital, Denmark, <sup>4</sup>Department of Gastroenterology & Hepatology, Aalborg Hospital, Denmark, <sup>5</sup>Institute of Medicine, University of Gothenburg, Sweden.

**Background and aims:** In patients with longstanding diabetes mellitus (DM) there is increasing evidence of abnormal processing of gastrointestinal sensations in the central nervous system. Using magnetic resonance (MR) diffusion tensor imaging, we aimed to characterize brain microstructure in areas involved in visceral sensory processing and correlated these findings to clinical parameters.

**Materials and methods:** Twenty-six patients with DM and gastrointestinal symptoms and 23 healthy controls were studied in a 3 Tesla MR scanner. Apparent diffusion coefficient (ADC) (i.e. diffusivity of water) and fractional anisotropy (FA) (i.e. organization of fibres) values were assessed in the “sensory matrix”: cingulate cortex, insula, prefrontal and secondary sensory cortex, amygdala and corona radiata. Corpus callosum served as control area.

**Results:** Patients had decreased FA values compared to controls in 1) anterior ( $P<0.001$ ), mid ( $P=0.002$ ) and posterior ( $P<0.001$ ) cingulate cortex, 2) prefrontal cortex grey matter ( $P<0.001$ ), 3) corona radiata ( $P<0.001$ ), 4) secondary sensory cortex ( $P=0.005$ ), 5) anterior white matter ( $P=0.048$ ), anterior grey matter ( $P=0.013$ ) and posterior grey matter ( $P<0.001$ ) insula. No difference between the two groups were found in corpus callosum ( $P>0.05$ ). The microstructural changes were for some areas correlated to clinical parameters such as bloating (anterior insula), mental wellbeing (anterior insula, prefrontal cortex, mid cingulate and corona radiata), autonomic function based on electrocardiographic results (posterior insula and anterior cingulate), and the presence of gastroparesis (anterior insula).

**Conclusion:** Our findings suggest that microstructural changes of brain areas involved in visceral sensory processing are correlated to autonomic dysfunction and might be involved in the pathogenesis of gastrointestinal symptoms in diabetes patients.



*Supported by: EU 7th Framework Programme and the Norwegian Diabetes Association*

## 47

### Severe symptomatic diabetic gastroparesis in type 1 and type 2 diabetes: ghrelin receptor agonist treatment improves symptoms

N. Ejskjaer<sup>1</sup>, R. Malik<sup>2</sup>, L. Tarnow<sup>3</sup>, P. Hellstrom<sup>4</sup>, G. Dimcevski<sup>5</sup>, J. Pezullo<sup>6</sup>, L. Shaughnessy<sup>6</sup>, R. Venuti<sup>6</sup>, P. Charlton<sup>6</sup>, G. Kosutic<sup>6</sup>, R. McCallum<sup>7</sup>

<sup>1</sup>Department of Medicine M (Endo+Diab), Aarhus University Hospital, Denmark, <sup>2</sup>Division of Cardiovascular Medicine, Manchester Royal Infirmary, UK, <sup>3</sup>Clinical Research Department, Steno Diabetes Center, Copenhagen, Denmark, <sup>4</sup>Department of Gastroenterology, Karolinska, Stockholm, Sweden, <sup>5</sup>Department of Gastroenterology, Haukeland University Hospital, Bergen, Norway, <sup>6</sup>Tranzyme Ltd, Durham, USA, <sup>7</sup>Health Sciences Center, Texas Tech University, Lubbock, USA.

**Background and aims:** Diabetic gastroparesis without symptoms is highly prevalent in both Type 1 and Type 2 diabetes, but is fortunately rare in its most severe form encompassing constant nausea, vomiting, postprandial fullness and bloating. The aim of this study was to characterize the effects of a ghrelin receptor agonist on symptoms of gastroparesis and to determine whether type 1 and type 2 diabetic patients responses are different.

**Materials and methods:** 92 patients {females 65%; age  $49.9 \pm 11.9$  years; 91% Caucasian; BMI  $28.8 \pm 5.1$ ; 60% type 1; HbA1c  $8.3 \pm 1.5\%$ ; PAGI-SYM  $3.0 \pm 0.8$ ; breath test t1/2 GE  $193 \pm 51$  min} suffering delayed gastric emptying and upper gastrointestinal symptoms were randomized to 10, 20 or 40 mg ghrelin receptor agonist or placebo. Symptoms were evaluated by patient-reported symptom severity scales (0-5) on days 8, 15, 28 (treatment) and 42, 58 (follow up). Effects were assessed by changes in symptom scores from baseline compared to end of treatment

**Results:** Significant improvements versus placebo were observed in individual symptoms across all ghrelin receptor agonist dose groups with a maximum improvement at the 20mg dose. Importantly, significant improvement vs. placebo was observed with 20mg ghrelin receptor agonist for each of prevalent symptoms for this patient population; magnitude of the effects was similar in type 1 and type 2 diabetic patients.

**Conclusion:** In our study ghrelin receptor agonist treatment significantly reduced symptoms of severe diabetic gastroparesis in both type 1 and type 2 diabetic patients.

*Supported by: Tranzyme Ltd, Durham, NC, USA*

## 48

### Initial treatment with duloxetine or pregabalin in patients with painful diabetic neuropathy. Data from the randomised, double-blind, parallel-group COMBO-DN Study

S. Wilhelm<sup>1</sup>, T. Tölle<sup>2</sup>, D. Bouhassira<sup>3</sup>, S. Perrot<sup>4</sup>, E. Kosek<sup>5</sup>, J.A. Micó<sup>6</sup>, A. Lledo<sup>7</sup>, R. Freynhagen<sup>8</sup>, G. Cruccu<sup>9</sup>, M. Späth<sup>10</sup>, V. Skljarevski<sup>11</sup>, A. Schacht<sup>1</sup>, S. Tesfaye<sup>12</sup>

<sup>1</sup>Lilly Deutschland, Bad Homburg, Germany, <sup>2</sup>Neurologische Klinik und Poliklinik, Technische Universität, München, Germany, <sup>3</sup>Centre d'Évaluation et de Traitement de la Douleur, Hôpital Ambroise Paré, Boulogne Billancourt, France, <sup>4</sup>Médecine Interne, Service de Rhumatologie, Hôpital Hotel Dieu, Université Paris V Descartes, France, <sup>5</sup>Karolinska Institutet, Stockholm, Sweden, <sup>6</sup>Grupo de Investigación y Desarrollo en Neuropsicofarmacol., Universidad de Cadiz, Spain, <sup>7</sup>Departamento de Neurología, Clínica Creu Blanca, Barcelona, Spain, <sup>8</sup>Benedictus Krankenhaus, Tutzing, Germany, <sup>9</sup>Sapienza University, Roma, Italy, <sup>10</sup>Spital Linth, Uznach, Switzerland, <sup>11</sup>Lilly Research Laboratories, Indianapolis, USA, <sup>12</sup>Royal Hallamshire Hospital, Sheffield, UK.

**Background and aims:** There have been no large head-to-head or combination treatment trials in painful diabetic peripheral neuropathic pain (DPNP). The COMBO-DN study primarily investigated the combination of duloxetine (DLX) and pregabalin (PGB) in patients with DPNP not responding to standard doses of each drug. The study also included for the first time, a randomized, double-blind, parallel group comparison of DLX and PGB for initial pain therapy.

**Materials and methods:** DPNP patients with a daily pain score of  $\geq 4$  on the Brief Pain Inventory Modified Short Form (BPI-MSF) were randomly assigned to 8-week initial treatment with either 60 mg DLX/day or 300 mg PGB/day. Efficacy was assessed by the BPI-MSF, Neuropathic Pain Symptom Inventory (NPSI), and Hospital Anxiety and Depression Scale (HADS). Scores were compared using Mixed Model Repeated Measures analyses or analyses of covariance, response rates ( $\geq 50\%$  reduction in BPI-MSF average pain after 8 weeks) by the Cochran-Mantel-Haenszel test. Frequency of

treatment-emergent adverse events (TEAEs) as safety measure was compared using Fisher's exact test.

**Results:** Of 1074 patients screened, 404 were randomized to DLX and 407 to PGB. After 8 weeks of treatment, differences between DLX and PGB were statistically significant in favor of DLX for all BPI-MSF scores, the NPSI total score, and all HADS scores. The least square (LS) mean (standard error [SE]) change from baseline in BPI-MSF average pain was -2.3 (0.11) for DLX vs. -1.7 (0.11) for PGB ( $p<0.001$ ); LS mean (SE) changes in NPSI total score were -19.4 (0.98) vs. -14.7 (0.98) ( $p<0.001$ ), and in HADS total score -3.1 (0.26) vs. 2.1 (0.26), respectively ( $p<0.005$ ). Response rates were 40.3% for DLX vs. 27.8% for PGB ( $p<0.001$ ). Most common TEAEs ( $>10\%$  of all patients) were dizziness (7.2% [DLX] vs. 15.1% [PGB];  $p<0.001$ ), somnolence (10.0% [DLX] vs. 10.9% [PGB];  $p>0.50$ ) and nausea (14.2% [DLX] vs. 6.5% [PGB];  $p<0.001$ ). However, the proportions of patients who discontinued due to TEAEs were not different between both drugs (11.5% [DLX] vs. 12.4% [PGB];  $p>0.50$ ).

**Conclusion:** The COMBO-DN study was the first ever trial directly comparing standard doses of DLX and PGB in DPNP patients. DLX (60 mg/day) was shown to have superior efficacy compared to PGB (300 mg/day). No new safety concerns were identified.

Clinical Trial Registration Number: NCT01089556

Supported by: Eli Lilly & Company

## OP 09 The effects of interventions in reality

49

### Effectiveness of oral antidiabetic drugs in treatment naïve patients: trends in HbA<sub>1c</sub> in the UK

A. Maguire<sup>1</sup>, B. Mitchell<sup>2</sup>;

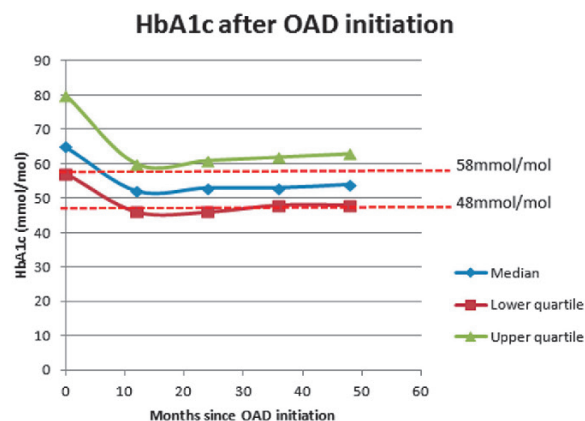
<sup>1</sup>Epidemiology and Database Analytics, UBC, London, UK, <sup>2</sup>Global Outcomes, Eli Lilly, Indianapolis, USA.

**Background and aims:** In the UK, oral antidiabetic therapy (OAD) is indicated for type 2 diabetes (T2D) if life style counselling is ineffective and HbA<sub>1c</sub> remains over 48mmol/mol (6.5%). In 2004, the introduction of the Quality and Outcomes Framework incentivised the systematic recording of measures such as HbA<sub>1c</sub>. The subsequent period has also coincided with increased level of computerised linkage with laboratories. Hence, given the accrued data it is now feasible to evaluate the changes in HbA<sub>1c</sub> over time and provide an insight into the effectiveness of initiating OAD which is the aim of this study.

**Materials and methods:** This is a retrospective cohort study of all new users of OAD between January 2006 and February 2011 identified in primary care practices that participate in the General Practice Research Database (GPRD). The most recent HbA<sub>1c</sub> value measured during the 6 months prior to OAD initiation was assigned as the baseline HbA<sub>1c</sub>. HbA<sub>1c</sub> values were assigned to each year following OAD initiation by selecting the HbA<sub>1c</sub> recorded closest to each time point (i.e., 12, 24, 36 and 48 months after OAD initiation) provided it was measured within 6 months of the time point. HbA<sub>1c</sub> is expressed in mmol/mol; Q1 and Q3 are the lower and upper quartiles.

**Results:** There were 63060 patients initiating OAD in the study period; mean age 62 years, 57% were male and 88% started on metformin. Median follow-up was 729 days (range: 1 to 1879 days). The median HbA<sub>1c</sub> at OAD initiation was 65 (Q1=57; Q3=80; N=43863) which reduced to 52 (Q1=46; Q3=60; N=34737) after one year. The HbA<sub>1c</sub> at subsequent time points was 53 (Q1=46; Q3=61; N=22650) at 2 years; 53 (Q1=48; Q3=62; N=12631) at 3 years; and 54 (Q1=48; Q3=63; N=4383) at 4 years. The mean within patient reduction was 15.8 (Standard Error "SE"=0.1) at one year. There was a slight increase in the within patient change in HbA<sub>1c</sub> for each subsequent year with respect to the previous year's measurement: 0.6 at 2 years (SE=0.1); 0.6 at 3 years (SE=0.1); 0.5 at 4 years (SE=0.2). Despite this reduction most patients remained above the recommended 48 mmol/mol (67% at 12months increasing to 74% at 4 years); similarly 28% were above 58 mmol/mol at 1 year since initiating OAD and 34% at 4 years. Patients with HbA<sub>1c</sub>>58 after 1 year were younger (59 vs. 63 years) and there was a slightly higher proportion of men (61% vs. 56%). Changes in treatment regimen were related to HbA<sub>1c</sub> at one year such that mean HbA<sub>1c</sub> was highest in patients who were augmented with insulin (mean=86) and lowest in patients who maintained their initial regimen (mean=52) or who discontinued therapy (mean=53).

**Conclusion:** This study in a large representative sample of patients with T2D in the UK has shown that there is a substantial reduction in HbA<sub>1c</sub> following OAD initiation. However, most patients remain above recommended levels and there is evidence of a slight increase of HbA<sub>1c</sub> over time. Changes in treatment regimen occur when levels are very high; over half the patient who augmented OAD with insulin had HbA<sub>1c</sub> that were above 80 mmol/mol at 12 months after initiating OAD.



Supported by: Eli Lilly



## 50

**The application of genome-wide association data to determine heritability of glycaemic response to metformin**

K. Zhou, C.N.A. Palmer, E.R. Pearson;  
Medical Research Institute, Dundee, UK.

**Background and aims:** Glycaemic response to metformin varies considerably among type 2 diabetes patients. Although robustly replicated loci for metformin response, such as that at the ATM locus, have been discovered it is not known how heritable the trait is. As for most drug response phenotypes, adopting the traditional twin or family study design to estimate metformin heritability is impractical because of the need to have relatives treated with the same drug. Here we report the first estimation of metformin glycaemic response heritability using a method based on genome-wide association data from unrelated patients.

**Materials and methods:** We used 3736 patients from the GoDARTS cohort who were genotyped by the WTCCC2 on the Affymatrix 6 array for pharmacogenomic investigations. Glycaemic response to metformin, measured as the maximum HbA1C reduction achieved within 18 months of treatment start, was definable in 1289 patients. Genetic relationship matrix was estimated with software GCTA using genotypes from 705125 autosomal SNPs. Further filtering was performed to ensure no pairwise genetic relationship  $>0.025$  among these seemingly independent individuals. Heritability was then estimated in a restricted maximum likelihood analysis, with clinical covariates such as baseline HbA1C, adherence and creatinine clearance included as fixed effect linear covariates.

**Results:** Our benchmark studies within the GoDARTS data estimated heritability for height as 28% (S.E.=10%;  $p=0.003$ ;  $n=3373$ ) and age at diagnosis of type 2 diabetes as 25% (S.E.=14%;  $p=0.04$ ;  $n=2497$ ). In the 1117 independent metformin treated patients, 36% (S.E.=32%,  $p=0.15$ ) of glycaemic response variation can be explained by autosomal SNPs.

**Conclusion:** Although more samples are required to quantify the heritability more accurately, our result suggests that metformin glycaemic response is likely to have a strong genetic component, on a par or greater than that seen with height or age of onset of type 2 diabetes.

Supported by: Sir Henry Wellcome Fellowship to KZ

## 51

**Metformin and other glucose-lowering treatments: risks and benefits. Nationwide epidemiological study**

E. Nils<sup>1</sup>, L. Schiöler<sup>2,3</sup>, A.-M. Svensson<sup>2</sup>, K. Eeg-Olofsson<sup>1</sup>, J. Miao Jonasson<sup>2</sup>, B. Zethelius<sup>4</sup>, J. Cederholm<sup>5</sup>, S. Gudbjörnsdottir<sup>1,2</sup>, B. Eliasson<sup>1</sup>;

<sup>1</sup>Department of Medicine, Sahlgrenska Academy at the University of Gothenburg, <sup>2</sup>Center of Registers in Region Västra Götaland, Gothenburg, <sup>3</sup>Department of Public Health and Community Medicine, Sahlgrenska Academy at the University of Gothenburg, <sup>4</sup>Department of Public Health and Caring Sciences/Geriatrics, Uppsala University, <sup>5</sup>Department of Public Health and Caring Sciences/Family Medicine and Clinical Epidemiology, Uppsala University, Sweden.

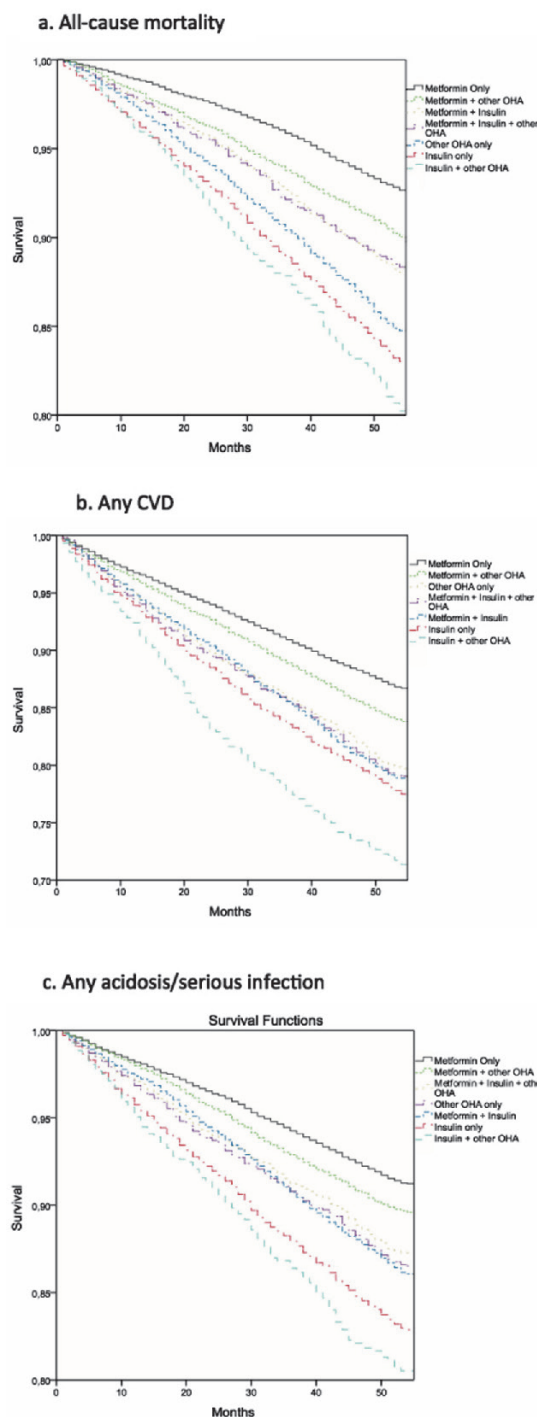
**Background and aims:** The long-term effectiveness and safety of glucose-lowering medications are under debate. This observational study from the Swedish National Diabetes Register (NDR) evaluates the risks of cardiovascular disease (CVD), lactic acidosis, serious infections and mortality in a large sample of pharmacologically treated patients with type 2 diabetes (DM2) in clinical practice, with particular emphasis on metformin.

**Materials and methods:** Pharmacologically treated DM2 patients registered in the NDR July 1, 2004 - December 31, 2007 were included ( $n=51\,675$ ), and grouped based on treatments. Risks of fatal/non-fatal CVD, acidosis/serious infection and all-cause mortality were analysed in all patients and in subgroups with different estimated glomerular filtration rate (eGFR) intervals. Mean follow-up was 3.9 years, equivalent to more than 200 000 patient-years at risk.

**Results:** Insulin monotherapy showed increased risks of fatal/non-fatal CVD and all-cause mortality compared to metformin monotherapy, hazard ratios (HR) 1.18 (95 % confidence limits: 1.07-1.29) and 1.34 (1.19-1.50), after adjustment with a propensity score including clinical characteristics, risk factors and treatments. In subgroup analyses, adjusted for covariance with similar covariates, metformin was not associated with increased risk of any of the outcomes in patients with eGFR 30-45, 45-60, or  $>60$  mL/min/1.73 m<sup>2</sup> compared to all other hypoglycaemic agents. In the subgroup with eGFR 45-60 mL/min/1.73 m<sup>2</sup>, metformin showed a reduced risk of any acidosis/serious infection, HR 0.85 (0.74-0.97), and all-cause mortality, 0.87 (0.77-0.99).

**Conclusion:** Metformin was associated with reduced risks of severe end-points, using propensity score or covariance adjustment. Results were consistent in patients with renal impairment, with no increased risk of acidosis/serious infection. In clinical practice, the benefits of metformin use clearly outbalance the risk of severe side effects. These results support the less strict approach to metformin use in patients with renal impairment (eGFR 45-60 mL/min/1.73 m<sup>2</sup>), advocated in most guidelines and imply further investigation with randomized clinical trials.

**Figure 1. Time (months) to event of all-cause mortality (a), any CVD (b), and any infection/acidosis (c) in each treatment group, unadjusted**



Supported by: The Region Västra Götaland, Local Authorities, BMS France

## 52

**Risk of acute coronary syndrome differs with glyburide and gliclazide treatment in patients with type 2 diabetes and ischaemic heart disease: a population-based cohort study**A. Abdelmonem<sup>1</sup>, D.T. Eurich<sup>2</sup>, J.-M. Gamble<sup>3</sup>, J.A. Johnson<sup>2</sup>, J. Seubert<sup>1,4</sup>, W. Qiu<sup>2</sup>, S.H. Simpson<sup>1</sup>;<sup>1</sup>Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, <sup>2</sup>School of Public Health, University of Alberta, Edmonton,<sup>3</sup>School of Pharmacy, Memorial University of Newfoundland, St. John's,<sup>4</sup>Department of Pharmacology, Faculty of Medicine, University of Alberta, Edmonton, Canada.

**Background and aims:** Sulfonylureas can inhibit cardiac KATP channels and block ischemic preconditioning, an endogenous protective mechanism in the heart provoked by prior ischemic events. At usual therapeutic doses glyburide is more likely to inhibit cardiac KATP channels than gliclazide. Since patients with a history of ischemic heart disease (IHD) are more likely to experience ischemic preconditioning, we hypothesized these patients would be more susceptible to adverse cardiovascular effects if they are using glyburide rather than gliclazide.

**Materials and methods:** Using administrative health records from Alberta, Canada, we conducted a population-based retrospective cohort study among patients with a history of IHD using either glyburide or gliclazide as their sole sulfonylurea between 1998 and 2008. Patients were followed until reaching the primary outcome of an acute coronary syndrome (ACS) event (composite of an ACS-related hospitalization or ACS-related mortality), leaving the province or December 31, 2008. Poisson regression analyses were used to estimate the incident rate ratio (IRR) of an ACS event, accounting for time-varying exposure to glyburide or gliclazide and assuming an 80% adherence rate.

**Results:** We identified 7,166 patients using glyburide and 6,367 patients using gliclazide, of whom 2,400 (17.7%) experienced an ACS event. Glyburide and gliclazide users were similar in mean age (71.6 vs. 71.2 years) and sex (58.5% vs. 58.2% men), but glyburide users had a longer mean follow-up time (4.3 vs. 3.6 years). Compared to gliclazide use, glyburide use was associated with a significantly higher incident rate of ACS events (12.7 vs. 10.5 events/1000 person-years; IRR: 1.20, 95% confidence interval (CI) 1.08 - 1.33). After controlling for concurrent drug therapies and co-morbidities, glyburide use remained associated with a significantly greater risk of ACS event compared to gliclazide (adjusted IRR: 1.18, 95% CI 1.07 - 1.31).

**Conclusion:** Patients with type 2 diabetes and a history of IHD are more likely to experience adverse cardiovascular events if they are using glyburide rather than gliclazide. These observations support the premise that the adverse cardiovascular effects of sulfonylureas are mediated by blockade of ischemic preconditioning. Moreover, selectivity sulfonylureas for cardiac KATP channels might explain this difference in cardiovascular risk.

*Supported by: Canadian Diabetes Association*

## 53

**The treatment of permanent neonatal diabetes secondary to Kir6.2 mutations with sulphonylureas: 5 year follow-up study**R.P. Finn, S.E. Flanagan, A.J. Chakera, A.T. Hattersley;  
Peninsula Clinical Research Facility, University of Exeter, UK.

**Introduction:** Kir6.2 mutations are identified in a third of patients with permanent neonatal diabetes, resulting in transfer to oral sulphonylureas in most cases. However, long term data on the efficacy, dosage requirement and side effect profile of their use in this population is lacking.

**Materials and methods:** We performed a retrospective follow-up study of 33 patients with permanent neonatal diabetes secondary to Kir6.2 mutations who transferred to sulphonylureas more than five years ago. Data was collected on glycaemic control, sulphonylurea dosage, neurodevelopmental features, side effects, height and weight at yearly intervals by the patients' clinicians. Data was collated and statistical analysis was performed using parametric paired T tests and non-parametric Mann-Whitney U tests

**Results:** Mean HbA1c on insulin therapy pre-transfer to sulphonylureas was 72mmol/mol (range 51 to 119). All patients had been successfully transferred to glibenclamide. HbA1c dropped to 46mmol/mol at six months following transfer to sulphonylureas. Treatment effect was sustained at five years, with a mean HbA1c of 44mmol/mol (range 34 to 56) at final five-year follow-up ( $p<0.0001$ ). Glibenclamide dose requirement reduced over time; average dose on transfer was 0.82mg/kg/day (range 0.1 to 2.02); average dose at final follow-up was 0.32mg/kg/day (range 0.06 to 0.77) ( $p<0.0001$ ). Neurodevel-

opmental features were noted to improve in seven out of the nine cases with neurodevelopmental features as part of the Developmental Delay, Epilepsy, and Neonatal Diabetes (DEND) syndrome spectrum. The two patients whose neurological features did not improve had been transferred to sulphonylureas post-puberty. Side effects such as mild hypoglycaemia and transient diarrhoea were noted in 3 cases, but were successfully managed with minor dosage adjustments. There were no adverse effects on patient growth.

**Conclusion:** This study demonstrates that sulphonylureas are effective and safe in the management of neonatal diabetes secondary to Kir6.2 mutations at 5 year follow-up. Superior glycaemic control is achieved with sulphonylureas compared to insulin treatment in this population. Treatment effect is sustained with reduced dosages of sulphonylureas required. This is in contrast to type 2 diabetes, where the early efficacy in glycaemic control achieved by sulphonylureas is not sustained at long term follow-up, and increased treatment doses are required. There is no adverse effect on paediatric growth and associated neurological features may be improved by treatment with sulphonylureas.

*Supported by: Peninsula Clinical Research Facility, SW Peninsula Deanery*

## 54

**Leisure time physical activity and risks of cardiovascular disease and mortality in type 2 diabetes: report from the Swedish national diabetes register**B. Zethelius<sup>1</sup>, S. Gudbjörnsdottir<sup>2</sup>, K. Eeg-Olofsson<sup>2</sup>, B. Eliasson<sup>2</sup>, J. Cederholm<sup>1</sup>;<sup>1</sup>Uppsala University, <sup>2</sup>Gothenburg University, Sweden.

**Background and aims:** Prior research has found a link between leisure time physical activity (LTPA) and cardiovascular disease (CVD) with observed preventive effects of increased LTPA. We assessed the association of level of LTPA with CVD and all cause mortality, taking risk factors for CVD into account, in patients with type 2 diabetes (T2DM) from the Swedish National Diabetes Register, NDR.

**Materials and methods:** 19,133 female (43%) and male (57%) patients aged 30-72 years, BMI  $\geq 18$  kg/m<sup>2</sup>, plasma creatinine  $\leq 150$   $\mu$ mol/l and free from CVD, congestive heart failure and atrial fibrillation at baseline (BL) in 2004-6 with follow-up until December 2009 (mean follow-up 4.8 years, 66,235 person years at risk). Five levels of LTPA were registered at BL and at end of study (EOS): never (level 1), less than 1 time weekly (level 2), 1-2 times weekly (level 3), regular 3-5 times weekly (level 4), or daily (level 5). Patients were divided into two groups: Group A (n=8,771) with lower BL-LTPA (levels 1, 2 or 3) and Group B (n=10,362) with higher BL-LTPA (levels 4 or 5) and further into two groups: Group C (n=5,675) with low BL-LTPA and low LTPA at EOS (levels 1, 2 or 3) and Group D (n=13,458) with either low BL-LTPA (levels 1, 2 or 3) or regular/higher BL-LTPA (levels 4 or 5) and with regular/higher LTPA at EOS (levels 4 or 5) taking increased LTPA into account. Hazard ratios (HR) with 95% confidence intervals (CI) were estimated using Cox regression with Group A and Group C as references, respectively, adjusting Model 1 (M1) for age, gender, diabetes duration, type of hypoglycaemic treatment, smoking and Model 2 (M2) by stratification by deciles of a propensity scores including the same covariates as M1 plus BMI, systolic blood pressure, HbA1c, LDL-cholesterol, HDL-cholesterol, triglycerides and degree of albuminuria at BL.

**Results:** HRs, CIs and p-values for: fatal and nonfatal CVD combined (n=987); fatal CVD (n=187); all cause mortality (n=656) were, respectively: for group B with A as reference;

M1: 0.83 (0.73-0.94)  $p=0.003$ ; 0.64 (0.48-0.86)  $p=0.003$ ; 0.73 (0.62-0.85)  $p<0.001$  and

M2: 0.90 (0.79-1.02)  $p=0.09$ ; 0.73 (0.54-0.98)  $p=0.03$ ; 0.78 (0.67-0.92)  $p=0.002$  and

for group D with C as reference;

M1: 0.58 (0.51-0.66)  $p<0.001$ ; 0.43 (0.32-0.58)  $p<0.001$ ; 0.50 (0.43-0.59)  $p<0.001$  and

M2: 0.62 (0.55-0.71)  $p<0.001$ ; 0.49 (0.36-0.66)  $p<0.001$ ; 0.53 (0.45-0.62),  $p<0.001$ .

**Conclusion:** Level of LTPA is related to fatal CVD and all cause mortality independent of conventional CVD risk factors in T2DM. Increased LTPA level during follow-up lowered CVD risk and mortality in this observational study on T2DM patients. Translating observational data into preventive care in individual T2DM patients has to be done with good judgement.



## OP 10 Type 2 diabetes: from risk models to survival

55

### Predictive factors of mortality among type 2 diabetic patients: do not forget clinical examination!

A. Sultan<sup>1</sup>, J. Daures<sup>2</sup>, C. Piot<sup>3</sup>, D. Mariano-Goulart<sup>4</sup>, A. Avignon<sup>1</sup>;  
<sup>1</sup>Nutrition-Diabetology, CHU Lapeyronie, <sup>2</sup>Biostatistic, IURC, <sup>3</sup>Cardiology, CHU Arnaud de Villeneuve, <sup>4</sup>Nuclear Medicine, CHU Lapeyronie, Montpellier, France.

**Background and aims:** The incidence of diabetes mellitus has increased at an alarming rate over the past 2 decades. The association between diabetes and cardiovascular diseases is well established, as still the main cause of death among these patients. We followed a cohort of 703 high risk type 2 diabetic patients during a median follow up of 5,8 years and thought to determine predictive factors associated with global mortality and cardiovascular mortality.

**Materials and methods:** Survival analysis was made. For univariate analysis, we used Kaplan Meyer methods. Covariables with p-value.

**Results:** Among these patients, 58% were male, mean age of 62,06 years, with a mean length of diabetes of 13,80 years and a HbA1c of 8,35%. 29% had positive microalbuminuria or proteinuria, 14% had a creatinin clearance inferior to 60 ml/min. 12% had obliterating arteriopathy of the lower limbs defined by pulse abolition noticed by 2 differents physicians. Among cardiovascular risk factors, mean LDL was 1,10 g/l, mean HDL was 0,53 g/l and 54% of these patients were under statin treatment. 60% were classified as having hypertension, and 43% were currents smokers. During follow up, 67 patients died (9,5%) with mainly cardiovascular death (57 patients). In multivariate analysis, obliterating arteriopathy of the lower limbs, insulin treatment, antiagregant treatment use, triglyceride level above 1,5 g/l were associated with global mortality. Obliterating arteriopathy of the lower limbs was the strongest predictor of mortality (RR=2,68; IC95%:1,58-4,52). Regarding predictive factors associated with cardiovascular mortality, we found that obliterating arteriopathy of the lower limbs, creatinin clearance under 60ml/min and high systolic blood pressure were associated. Once again, obliterating arteriopathy of the lower limbs was the strongest predictor of cardiovascular mortality (RR=2,79; IC95%:1,68-4,64).

**Conclusion:** In conclusion, presence of obliterating arteriopathy of the lower limbs diagnosed by clinical examination was the strongest factor associated with global and cardiovascular mortality in high risk type 2 diabetic patients. This should lead us to intensify risk factors treatment in this group of patients and maybe to carefully discuss coronarography exploration.

56

### Comparison of predictability of cardiovascular estimation risk models in Greek diabetic patients

N. Melas, A. Kamaratos, K. Mpotsios, M. Sevdalis, K. Tzirogiannis, C. Verras, G. Panoutsopoulos, P. Gavra, A. Melidonis;  
 Diabetes Center, Tzanio General Hospital, Piraeus, Greece.

**Background and aims:** Objective of this prospective study is the comparison of the predictability of two established cardiovascular estimation risk engines, UKPDS (UK Prospective Diabetes Study) Risk Engine and HeartScore (Systematic Coronary Risk Evaluation), in diabetic patients.

**Materials and methods:** This prospective study was conducted in the Diabetes Center of Tzanio General Hospital in the period 1998 - 2001. The study population included a thousand eighty four (n=1084) diabetic patients. They have been observed for ten years regarding two cardiovascular end points, fatal coronary heart disease (CHD) and fatal stroke. The initial data was inputted individually on both of the risk engines, generating rates of estimation of cardiovascular risk. These rates were compared with the real outcome data after 10 years follow up of these patients. ROC curves was used in order to identify the predictability of the two models, comparing the estimated outcomes calculated in the onset of the study and the real outcomes recorded on the conclusion of the study after 10 years follow up.

**Results:** The study sample included 1084 diabetic patients (498 M / 586 F) with mean age of 60 years for both of the sex groups and mean diabetes duration of 6.9 years for the males and 8.2 years for the females. The comparison of the predictability of the two risk engines was completed for the totality of

the sample and by the gender individually. For the total of the study sample, the mean value ( $\pm$  SD) of the estimated risk of fatal event (CHD and stroke) was  $17.95 \pm 0.48$  for the UKPDS and  $5.91 \pm 0.14$  for the HeartScore risk engine. The real outcome was 130 total fatal events (68 CHD and 52 Stroke). Using the ROC curves we observed that in UKPDS the area under the curve (AUC) was 0.831 (95% CI, 0.794 - 0.867,  $p < 0.001$ ) while in HeartScore was 0.782 (95% CI, 0.747 - 0.818,  $p < 0.001$ ). Comparing the risk engines by gender individually we observed that the mean value of the estimated risk of fatal event in women group was  $13.84 \pm 0.52$  for the UKPDS and  $4.15 \pm 0.12$  for the HeartScore risk engine. The real outcome in this group was 42 fatal events (20 CHD and 22 Stroke). In the same group in UKPDS the AUC was 0.794 (95% CI, 0.735 - 0.852,  $p < 0.001$ ) while in HeartScore was 0.783 (95% CI, 0.735 - 0.832,  $p < 0.001$ ). In men group, the real outcome was 78 fatal events (48 CHD and 30 Stroke) while the mean value of the estimated risk of fatal event was  $22.81 \pm 0.78$  for the UKPDS and  $8.00 \pm 0.23$  for the HeartScore risk engine. In men group we observed that in UKPDS the AUC was 0.841 (95% CI, 0.796 - 0.887,  $p < 0.001$ ) while in HeartScore was 0.747 (95% CI, 0.694 - 0.799,  $p < 0.001$ ).

**Conclusion:** Comparing two established cardiovascular estimation risk engines in a large sample of diabetic patients after 10 years follow up, we observed that the prediction ability of fatal cardiovascular event (CHD and stroke) of UKPDS overmatches against HeartScore. We observed furthermore that the predictability of UKPDS overmatches against HeartScore in both genders individually, especially in men.

57

### Incidence of death, hospitalisation and new left ventricular dysfunction in type 2 diabetic patients. Two years follow-up in the DYDA study

C.B. Giorda<sup>1</sup>, E. Nada<sup>1</sup>, G. Vespasiani<sup>2</sup>, L. Bortolato<sup>3</sup>, D. Lucci<sup>4</sup>, A. Leopardi<sup>5</sup>, E. Zarra<sup>6</sup>, G. De Simone<sup>7</sup>, M. Comaschi<sup>8</sup>, on behalf of DYDA Investigators;  
<sup>1</sup>Diabetology Dept., Ospedale Maggiore, Chieri, <sup>2</sup>Diabetology Dept., Ospedale Madonna del Soccorso, San Benedetto del Tronto, <sup>3</sup>Medicine Dept., Ospedale Civile, Mirano, <sup>4</sup>Centro Studi ANMCO, Firenze, <sup>5</sup>Diabetology Dept., Ospedale S. Giovanni di Dio, Firenze, <sup>6</sup>Diabetology Dept., Spedali Civili, Brescia, <sup>7</sup>Clin Experiment Medicine Dept., AOU Federico II, Napoli, <sup>8</sup>Centro per il Piede Diabetico, ICLAS, Rapallo, Italy.

**Background and aims:** T2DM is cause of CV complications, atherosclerosis, heart failure and death. DYDA is a prospective, multicenter epidemiological study in 960 T2DM patients, aged >45 years and without overt cardiac diseases. Baseline echocardiographic examination revealed a high prevalence of preclinical LV systolic (21%) and diastolic dysfunction (27%) or both (12%) (LVD), measured by midwall shortening (MFS) and transmitral flow pattern, respectively, as previously reported. Patients were followed-up for 2 years. In the present study, we report data on echocardiographic re-evaluation and clinical events.

**Materials and methods:** Systolic LVD (DLVD) was defined as EF  $\leq 50\%$  or MFS  $\leq 15\%$ ; diastolic LVD (SLVD) was identified in all conditions different from "normal", defined as transmitral E/A ratio between 0.75 and 1.5 and E velocity deceleration time >140 msec. The primary outcome was a composite of major events, (all-cause death and hospital admissions). Secondary endpoint was the incidence of new LVD.

**Results:** Follow-up data were available on 957 patients. During the follow-up 15 deaths (1.6%, 3 CV death, 11 non CV death, 1 of unknown etiology) and 181 hospital admissions were observed in 139 patients (48 for CV cause, 133 for non CV cause). In multivariate analysis, older age (67 vs 56 yrs OR 1.41, 95% CI 1.05-1.88), high LDL (134 vs 93 mg/dL OR 1.39, CI 1.08-1.78), low HDL (57 vs 42.5 mg/dL OR 0.76, CI 0.60-0.98), high Hb1Ac (7.6 vs 6.0% OR 1.3, CI 1.05-1.62), peripheral arterial disease (OR 3.49, CI 1.54-7.9) and treatment with repaglinide (OR 2.01, CI 1.17-3.46) were independently associated with a major event. LVD was observed at baseline or during follow-up in 88.1% (616/699) of patients; a systolic LVD in 63.9% (338/529), and a diastolic LVD in 66.5% (463/696). In a multivariate analysis, older age (67 vs 56 yrs OR 2.45, 95% CI:1.86-3.23), high Hb1Ac (7.6 vs 6.0% OR 1.25, 95% CI:1.01-1.54), high heart rate (80 vs 68 bpm OR 1.23, 95% CI: 1.03-1.47) and high DBP (90 vs 78 mmHg OR 2.29; 95% CI: 1.41-3.72) were independently associated with DLVD; whereas SLVD was associated only with waist circumference (106 vs 92 cm OR 1.39, 95% CI:1.05-1.84). New onset of systolic LVD (either EF  $< 50\%$  or MFS  $< 15\%$ ) was observed in 66/388 pts. (17.0%) and that of diastolic LVD in 126/572 pts (22.0%).

**Conclusion:** In patients with DM without overt cardiac disease at baseline, LVD is a frequent finding, and is associated with older age, higher HbA1c, heart rate, diastolic BP and waist. In these patients, all-cause death or hospi-

talisation occurred in nearly 16% of the cases at mid-term follow-up, being the great majority of them of non-CV reason. Independent predictors of such adverse clinical events were older age, pathologic lipid profile, poor control of DM, peripheral arteriopathy and repaglinide therapy.

*Supported by: an unrestricted grant from Sanofi-Aventis*

## 58

### Have insulin-treated diabetic patients, admitted with non-ST elevation segment acute coronary syndrome, a worst prognosis than those treated with an oral anti-diabetic?

J. Chin, P. Sousa, N. Marques, J. Silva, J. Amado, W. Santos, J. Mimoso, A. Lopes, E. Pina, A. Camacho, V. Brandão, I. Jesus;  
Hospital of Faro, Portugal.

**Background and aims:** There are questions about what is the best therapeutic strategy for patients (P) with diabetes mellitus (DM) who suffered a non-ST segment elevation acute coronary syndrome (NSTACS). The main question is on the benefits of use of insulin versus oral anti-diabetic for glycemic control in these P. The aim of this study was to determine if DM insulin-treated (IT) P admitted with NSTACS have a higher complications and mortality rates during hospitalization and after discharge, than those treated with oral anti-diabetics (OAD).

**Materials and methods:** We conducted a retrospective, descriptive and correlational study, based on a prospective registry, involving P admitted with NSTACS in a Cardiology Department between January/2006 to October/2010. We evaluated baseline characteristics, admission data, therapeutic strategy and a univariate and multivariate analyzes for hospital events - ventricular fibrillation (VF), complete atrioventricular block (BAVC), re-AMI (RE-MI), major bleeding (MB), stroke and in-hospital mortality - and after discharge - RE-MI, stroke, readmission for heart disease (RHD) and mortality (cardiovascular and overall). Statistical analysis was performed using SPSS 13.0.

**Results:** Of the 1086 P admitted for NSTACS, 357 P were diabetic and of these 50 P (14%) were on insulin therapy. The DM IT P had more frequently a history of peripheral arterial disease (PAD), (30% vs 12.1%,  $p=0.001$ ), coronary artery bypass graft (CABG) (24% vs 12.4%,  $p=0.028$ ) and left ventricular dysfunction at admission (42.9% vs 25.4%,  $p=0.011$ ). There were no significant differences in other baseline characteristics, admission data or therapeutic strategy. Regarding the in-hospital complications, there were no statistically significant differences between the two groups, even in the mortality (0% vs 0.7%,  $p=0.567$ ). The mean follow-up (FU) was  $41 \pm 16$  months (FU rate of 92%). The DM IT P had the similar complications events than OAD DM P - RE-MI (22.5% vs 20.6%,  $p=0.788$ ), stroke (2.5% vs 3.2%) and RHD (25% vs 30.6%,  $p=0.475$ ). They also presented a similar CVM (11.4% vs 8.8%,  $p=0.589$ ) and OM (27.3% vs 22.3%,  $p=0.462$ ).

**Conclusion:** 1. The diabetic patients insulin-treated admitted with NSTACS presented more frequently a history of PAD, CABG and left ventricle dysfunction, than those treated with oral anti-diabetics. 2. Prognosis during hospitalization and after discharge were similar for both groups.

## 59

### Diabetes mellitus in patients with non-ST segment elevation acute coronary syndrome - worse prognosis?

P. Sousa, N. Marques, J. Chin, J. Silva, J. Amado, W. Santos, A. Lopes, E. Pina, J. Mimoso, S. Pereira, V. Brandão, I. Jesus;  
Hospital of Faro, Portugal.

**Background and aims:** The aim of this study was to determine if diabetes mellitus (DM) patients (P) admitted in a Cardiology Department (CD) with non-ST segment elevation acute coronary syndrome (NSTACS) have higher rates of complications and mortality, during hospitalization and after discharge, compared with nondiabetics P. We also sought to determine the predictors of mortality in DM P with NSTACS.

**Materials and methods:** We conducted a retrospective, descriptive and correlational study, based on a prospective registry, involving P admitted with NSTACS between January 2006 to October 2010. We evaluated baseline characteristics, admission data, in-hospital events - ventricular fibrillation, complete atrioventricular block, re-infarction (RE-MI), major bleeding, stroke, mortality and follow-up (FU) events - RE-MI, stroke, readmission for heart disease (RHD) and mortality (cardiovascular - CVM and overall - OM)). Midterm monitoring was conducted by a cardiologist ( $41 \pm 16$  months, FU

rate 93%). We also performed an univariate and multivariate analysis, in DM P, of in-hospital mortality and mortality (CVM and OM) during FU. Statistical analysis was performed using SPSS 13.0.

**Results:** Of the 1086 P admitted with NSTACS, 357 (33%) had DM, of which 50 P (14%) were under insulin therapy. The DM P were mostly women ( $p=0.016$ ), hypertensive ( $p<0.001$ ), had dyslipidemia ( $p<0.001$ ), and were non-smokers ( $p<0.001$ ). More often had a history of stroke ( $p=0.005$ ), angina ( $p<0.001$ ), myocardial infarction ( $p<0.001$ ) and peripheral arterial disease (PAD) ( $p<0.001$ ). During hospitalization, there were no significant difference between the two groups concerning the complications and the mortality rates. The in-hospital mortality rate of the DM P was 0.6%. There were no independent predictors of mortality. During the FU, DM P presented more RE-MI (20.9% vs 8.7%,  $p<0.001$ ) and more RHD (29.8% vs 20.9%,  $p=0.004$ ). DM P had similar CVM (9.2% vs 7.1%,  $p=0.253$ ) but a higher OM (22.9% vs 16.2%,  $p=0.011$ ). In the DM P, the independent predictors of CVM were the female gender ( $p=0.011$ ), previous myocardial infarction ( $p=0.010$ ) and not performing percutaneous coronary intervention (PCI) ( $p=0.027$ ). The independent predictors of OM were a history of PAD ( $p=0.042$ ) and the left ventricle ejection fraction  $<30\%$  ( $p<0.001$ ).

**Conclusion:** 1. DM P had more cardiovascular risk factors for coronary disease. 2. During hospitalization, despite the higher risk associated to DM P, there were no differences regarding complications and mortality rates between the two groups. 3. There were no independent in-hospital mortality predictors, probably due a low mortality rate of the DM P. 4. After discharge, DM P had a higher risk of RE-MI and RHD, with a higher OM, at the expense on non-CVM. 5. In the DM P, the independent CVM predictors were female gender, previous myocardial infarction and not performing PCI. The independent predictors of OM were a history of PAD and left ventricle dysfunction.

## 60

### High 3-year-mortality rates in females with newly diagnosed diabetes after acute STEMI and NSTEMI: results of the SWEETHEART registry

D. Tschöpe<sup>1,2</sup>, F. Towae<sup>3</sup>, A. Papp<sup>4</sup>, E. Deeg<sup>5</sup>, J. Senges<sup>5</sup>, U. Zeymer<sup>3</sup>, R. Zahn<sup>3</sup>, P. Bramlage<sup>6</sup>, A.K. Gitt<sup>3,5</sup>;

<sup>1</sup>Stiftung „Der herzkrankte Diabetiker“, Stiftung in der Deutschen Diabetes-Stiftung, Bad Oeynhausen, <sup>2</sup>Universitätsklinik der Ruhr-Universität Bochum, Herz- und Diabeteszentrum, Bad Oeynhausen, <sup>3</sup>Medizinische Klinik B - Abteilung für Kardiologie, Klinikum der Stadt Ludwigshafen GmbH, <sup>4</sup>Med. Klinik B, Kardiologie, Herzzentrum Ludwigshafen, <sup>5</sup>Institut für Herzinfarktforschung Ludwigshafen an der Universität Heidelberg, Ludwigshafen, <sup>6</sup>Institut für Pharmakologie und präventive Medizin, Mahlow, Germany.

**Background and aims:** There is a considerable co-morbidity between coronary artery disease (CAD) and diabetes. Dysglycaemia is however frequently unknown and guidelines released by the ESC / EASD now recommend testing for diabetes using an oral glucose tolerance test (OGTT) in patients with established CAD.

**Materials and methods:** A total of 2,767 consecutive patients presenting with STEMI or NSTEMI were enrolled into SWEETHEART starting in 2007. Except for those with known diabetes all patients underwent an OGTT at day 4 after admission. The present analysis reports the prevalence of dysglycaemia according to gender and the impact of newly diagnosed diabetes on mortality at the 3-year follow-up.

**Results:** Female patients were older but had less prior myocardial infarction (MI) and percutaneous coronary interventions (PCI) than male patients. Known diabetes was more frequent (30.2 vs. 23.1%) and diabetes duration longer (10 vs. 7 years) in female patients as was the rate of newly diagnosed diabetes upon OGTT (19.7 vs. 15.3%). In contrast to men, 3-year mortality rates with established and newly diagnosed diabetes was virtually identical (30.0 and 30.5% respectively) in women (35.4 vs. 21.8% in men).

**Conclusion:** Female patients have substantially higher rates of known as well as newly diagnosed diabetes compared to their male counterparts when presenting with acute myocardial infarction. Mortality rates with newly diagnosed diabetes are substantially higher in female than in male patients at the 3 year follow-up.

	Female (n=706)	Male (n=2061)	p-value
Age (years)	71	64	<0.01
Prior MI (%)	13.0	18.8	<0.01
Prior PCI (%)	12.2	18.1	<0.01
Prior stroke (%)	5.7	5.6	0.96
Primary PCI (STEMI/NSTEMI) (%)	80.1	85.3	<0.01
Hospital mortality (%)	2.6	2.2	0.52
Known diabetes (%)	30.2	23.1	<0.01
Duration of diabetes (years)	10	7	<0.01
Results of OGTT			
Newly diagnosed diabetes	19.7	15.3	<0.01
IGT / IFG	18.1	23.1	<0.01

Clinical Trial Registration Number: NCT01197742

Supported by: Sanofi Aventis

## OP 11 Links between obesity, inflammation and insulin resistance

### 61

#### N-(Carboxymethyl)lysine-RAGE axis: a novel link between obesity, inflammation and insulin resistance

K. Gaens<sup>1</sup>, P.M.G. Niessen<sup>1</sup>, M.M.J. van Greevenbroek<sup>1</sup>, C.J.H. van der Kallen<sup>1</sup>, H.W.M. Niessen<sup>2</sup>, S.S. Rensen<sup>1</sup>, W.A. Buurman<sup>1</sup>, J.M. Greve<sup>1</sup>, G.H. Goossens<sup>1</sup>, E.E. Blaak<sup>1</sup>, M.A.M. van Zandvoort<sup>1</sup>, A. Bierhaus<sup>3</sup>, C.D.A. Stehouwer<sup>1</sup>, C.G. Schalkwijk<sup>1</sup>

<sup>1</sup>Maastricht University Medical Center, Netherlands, <sup>2</sup>Vrije Universiteit Medical Center, Amsterdam, Netherlands, <sup>3</sup>University of Heidelberg, Germany.

**Background:** Obesity is characterized by a dysregulated production and secretion of pro- and anti-inflammatory adipokines, which are critically involved in the pathogenesis of obesity-related complications such as insulin resistance and type 2 diabetes mellitus. However, the mechanisms underlying an abnormal expression of adipokines have not been fully elucidated. We hypothesized that accumulation of the advanced lipoxidation endproduct, N<sup>ε</sup>-Carboxymethyllysine (CML) in adipose tissue in obesity causes dysregulation of adipokines, thus contributing to the development of obesity-related insulin resistance.

**Methods:** Accumulation of CML in obese adipose tissue was assessed by immunohistochemistry. Plasma levels of CML were measured by UPLC-Tandem MS in controls (n=77), patients with familial combined hyperlipidemia (FCHL, n=42), and obese subjects (n=44). To study trapping of circulating CML in adipose tissue and to address the role of CML-RAGE axis in the dysregulation of inflammatory cytokines, receptor for advanced glycation endproduct (RAGE)-deficient obese mice (RAGE<sup>-/-</sup>/Lepr<sup>Db/-</sup> mice) were generated by crossing RAGE<sup>-/-</sup> with Lepr<sup>Db/-</sup> mice. The effect of CML on expression of inflammatory markers was also studied in human adipocyte cell culture. Finally, the role of CML-RAGE axis in the development of insulin resistance was investigated by performing glucose and insulin tolerance tests in RAGE<sup>-/-</sup>/Lepr<sup>Db/-</sup> and RAGE<sup>+/+</sup>/Lepr<sup>Db/-</sup> mice.

**Results:** We report a strong CML accumulation in adipose tissue in obesity, with significantly more accumulation in visceral than in subcutaneous adipose tissue (1.5±0.2 vs 1.3±0.2, p<0.001). Circulating CML levels were lower in obese subjects compared with controls (1.3±0.2μM vs 1.9±0.5μM, p<0.001), and were inversely related to visceral adipose tissue mass (r=-0.557, p<0.001), suggesting trapping of circulating CML in adipose tissue. Fluorescently labeled CML was trapped in adipocytes of obese Lepr<sup>Db/-</sup> mice, but not in RAGE<sup>-/-</sup>/Lepr<sup>Db/-</sup> mice. Moreover, inhibition of CML trapping in RAGE<sup>-/-</sup>/Lepr<sup>Db/-</sup> was associated with less inflammation (decreased pro-inflammatory cytokines IL-1β, IL-12, IL-6, IL-8, TNF-α, IFN-γ, and increased anti-inflammatory cytokines IL-10 and adiponectin). This demonstrates that RAGE is a key modulator of CML trapping and that CML-RAGE axis plays a major role in inducing inflammatory responses in obesity. Incubation of human adipocytes with CML also resulted in upregulation of pro-inflammatory markers, whereas anti-inflammatory markers decreased. Furthermore, we demonstrated that intervening with CML trapping and CML-RAGE mediated inflammatory responses were associated with improved glucose homeostasis in RAGE<sup>-/-</sup>/Lepr<sup>Db/-</sup> mice.

**Conclusion:** Our findings demonstrate the occurrence of RAGE-dependent CML trapping in adipose tissue in obesity, which contributes to the dysregulation of adipokines and development of obesity-related insulin resistance. Targeting this novel mechanism of altered production of adipokines may have therapeutic potential in the management of the obesity-associated complications.



## 62

**Inhibition of PSGL-1 ameliorates obesity-related insulin resistance by prevention of inflammation in adipose tissue and liver in db/db mice**D. Hirota<sup>1,2</sup>, K. Shikata<sup>1,3</sup>, C. Sato<sup>1</sup>, R. Kodera<sup>1,3</sup>, S. Miyamoto<sup>1</sup>, N. Kajitani<sup>1</sup>, T. Takatsuka<sup>1</sup>, D. Ogawa<sup>1</sup>, H. Kataoka<sup>1</sup>, H. Makino<sup>1</sup>;<sup>1</sup>Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine Dentistry and Pharmaceutical Sciences,<sup>2</sup>Diabetes Center, Okayama University Hospital, <sup>3</sup>Center for Innovative Clinical Medicine, Okayama University Hospital, Japan.

**Background and aims:** There have been accumulating evidences that inflammation in adipose tissue is involved in the mechanism of obesity-related insulin resistance. Macrophages and proinflammatory cytokines are increased in visceral adipose tissues of obese people and animal models. Infiltration of monocyte/macrophage is mediated by the interaction of cell adhesion molecules expressed on monocytes and endothelial cells. We previously screened the gene expression profiles in adipose tissues from obese mice using DNA microarray and found that P-selectin glycoprotein ligand-1 (PSGL-1) is up-regulated in both db/db mice and high-fat diet (HFD) fed mice. PSGL-1 is expressed on both leukocytes and endothelial cells and binds to P-, L- and E-selectin. We found PSGL-1 is expressed on both endothelial cells and macrophages in adipose tissues of obese mice. Furthermore, we demonstrated that PSGL-1 deficient mice fed with HFD revealed decreased macrophage infiltration into adipose tissues, improved adipocyte hypertrophy and insulin resistance as compared with wild type mice fed with HFD. The aim of this study is to clarify the effects of anti-PSGL-1 antibody on the adipose tissue and the liver in obese db/db mice.

**Materials and methods:** Anti-PSGL-1 monoclonal antibody or normal rat IgG was administered by intraperitoneal injection to six week-old male db/db mice. An intraperitoneal insulin tolerance test (ipITT) and an intraperitoneal glucose tolerance test (ipGTT) were performed at 7 weeks old. We measured serum lipids, insulin, HbA1c and adipocytokines. We also examined the gene expression of proinflammatory cytokines, chemokines, adhesion molecules, adipocytokines and infiltration of macrophages in epididymal white adipose tissues (WAT) and liver.

**Results:** There was no significant difference in body weight, weight of epididymal WAT and liver, total cholesterol, free fatty acid and HbA1c. Significant reductions were observed in fasting blood glucose and LDL cholesterol in treated group (treated group vs control group,  $p < 0.05$ ). The average values of triglyceride and fasting IRI were decreased in treated group as compared with control group. The size of adipocytes in epididymal WAT was also significantly decreased in treated group as compared with control group. Glucose tolerance and insulin sensitivity were significantly improved in treated group in ipITT and in ipGTT. The expressions of MCP-1, CD68 (treated group vs control group,  $p < 0.05$ ) and IP-10 (treated group vs control group,  $p < 0.01$ ) were decreased in the adipose tissue and the liver in treated group as compared with control group.

**Conclusion:** The administration with anti-PSGL-1 antibody revealed decreased macrophage infiltration not only in the adipose tissues but also in the liver in db/db mice. Anti-PSGL-1 antibody improved adipocyte hypertrophy and insulin resistance. These results provide the direct evidence that PSGL-1-selectin pathway promotes the recruitment of macrophages into the adipose tissue and the liver. PSGL-1 might be a novel target for the prevention of insulin resistance in obesity.

*Supported by: Grant-in Aid for Scientific Research from the Ministry of Education*

## 63

**The functional role of oncostatin M in the development of insulin resistance in mice**Y. Morikawa<sup>1</sup>, T. Komori<sup>1</sup>, M. Tanaka<sup>2</sup>, E. Senba<sup>1</sup>, A. Miyajima<sup>2</sup>;<sup>1</sup>Anatomy and Neurobiology, Wakayama Medical University, Wakayama,<sup>2</sup>Laboratory of Cell Growth and Differentiation, Institute of Molecular and Cellular Bioscience, The University of Tokyo, Japan.

**Background and aims:** Oncostatin M (OSM) is a member of the interleukin (IL)-6 family of cytokines and plays important roles in the variety of biological functions including the regulation of hematopoiesis, liver development, and inflammatory response. However, the relationship between OSM and metabolic disease remains unclear. In the present study, we investigated the roles of OSM in the adipose tissue inflammation and insulin resistance.

**Materials and methods:** We used OSMR $\beta$ -deficient (OSMR $\beta$ <sup>-/-</sup>) mice to examine the role of OSM in the development of insulin resistance. We also investigated the therapeutic effect of OSM on insulin resistance of ob/ob mice.

**Results:** OSMR $\beta$  was mainly localized in the adipose tissue macrophages (ATMs) of obese mice suggesting that OSM contributes to the development of adipose tissue inflammation by regulating the functions of ATMs. To test this possibility, we first analyzed adipose tissue inflammation and insulin resistance in OSMR $\beta$ <sup>-/-</sup> mice on a normal diet (ND). OSMR $\beta$ <sup>-/-</sup> mice showed insulin resistance, whereas there were no significant differences in the body weights between OSMR $\beta$ <sup>+/+</sup> and OSMR $\beta$ <sup>-/-</sup> mice. In ATMs of OSMR $\beta$ <sup>-/-</sup> mice, the ratio of M1/M2 macrophages was increased compared to that in OSMR $\beta$ <sup>+/+</sup> mice. In addition, the expression of TNF- $\alpha$  was increased, while the expression of IL-10 was decreased in the adipose tissue of OSMR $\beta$ <sup>-/-</sup> mice. Next, we analyzed adipose tissue inflammation and insulin resistance in OSMR $\beta$ <sup>-/-</sup> mice on a high fat diet (HFD). OSMR $\beta$ <sup>-/-</sup> mice fed a HFD exhibited higher insulin resistance than that in OSMR $\beta$ <sup>+/+</sup> mice. The increased expression of TNF- $\alpha$  and IL-10 was observed in OSMR $\beta$ <sup>-/-</sup> mice fed a HFD. To determine the direct effects of OSM on macrophages, we treated OSM to mouse peritoneal exudate macrophages (PEMs). The expression of IL-10 was increased by OSM in PEMs in addition to the activation of signal transducer and activator of transcription 3 and cAMP response element binding protein. In addition, the expression of M2 markers (arginase-1 and CD206) was increased in OSM-treated PEMs compared to those in vehicle-treated PEMs. These results indicate that OSM contributes to the development of insulin resistance by polarizing the phenotypes of macrophages to M2 with the induction of IL-10 expression. Finally, OSM was intraperitoneally injected into ob/ob mice to assess the effects of OSM on the insulin resistance of obese mice. Glucose intolerance and insulin resistance in ob/ob mice was improved by OSM. In addition, the expression of a marker of M1 macrophage (iNOS) and pro-inflammatory cytokine (TNF- $\alpha$ ) were decreased, while the expression of markers of M2 macrophage (CD206 and CD163) and anti-inflammatory cytokine (IL-10) were increased in the adipose tissue of OSM-injected ob/ob mice. These results suggest that OSM improves adipose tissue inflammation and insulin resistance during obesity.

**Conclusion:** OSM may play important roles in the development of adipose tissue inflammation and insulin resistance by changing the phenotypes of macrophages to M2 and inducing the expression of IL-10 directly.

*Supported by: Priority Areas from Wakayama Medical University*

## 64

**Interleukin 21 knockout protects from adipose tissue inflammation in a diet-induced obesity model**

M. Fabrizio, A. Marino, V. Casagrande, V. Marchetti, M. Mavilio, M. Cavallera, R. Menghini, F. Pallone, R. Lauro, G. Monteleone, M. Federici; Medical School, University of Rome Tor Vergata, Italy.

**Background and aims:** Interleukin-21, a pro-inflammatory cytokine, is mainly produced by multiple effector lymphocyte CD4<sup>+</sup> T and Natural Killer T cells. IL-21 receptors are widely expressed resulting to pleiotropic actions of IL-21 on multiple tissues. Recent studies suggested a role for IL-21 in mediating chronic inflammation. Obesity is accompanied by adipose tissue chronic low-grade inflammation, which promotes insulin resistance and type-2 diabetes. In particular regulatory T cells (Tregs) may play a critical role in modulating adipose tissue inflammation via interactions with both adaptive and innate immune system. Interestingly Tregs are highly enriched in the abdominal fat of lean mice but are specifically reduced in adipose tissue of obese animals. Because IL-21 is known to exert negative effects on Treg activity we hypothesized that it could play a role in obesity-induced insulin resistance.

**Materials and methods:** We compared C57BL/6J WT and IL-21 knockout (IL-21KO) mice in a context of diet induced obesity (DIO). Animals were subjected to metabolic characterization: weight and blood glucose monitoring, glucose and insulin tolerance tests, energy expenditure and activity rating. At the end of 20 weeks of DIO, WT and KO mice were sacrificed and metabolically active organs were collected for molecular and histological studies. Fasting and feeding sera were collected for assessment of the amount of insulin and NEFA.

**Results:** Adipocytes, stromal vascular cells and hepatocytes isolated from DIO wt mice showed increased levels of IL-21 mRNA compared to mice under standard chow diet, suggesting its involvement in developing or in maintaining the inflammatory condition associated to obesity. During DIO, IL-21 KO mice compared to wt littermates showed lower weight, characterized by reduced adiposity associated with decreased fat pad mass and adipocyte size ( $P < 0.0001$ ), lower levels of blood glucose ( $p < 0.005$ ) and hypoinsulinemia

( $p < 0.05$ ). Metabolic differences were observed mostly in the fasting state suggesting an involvement of IL-21 in adaptation to fasting. WAT (white adipose tissue) of IL-21 KO mice showed higher levels of IRF4 ( $P < 0.005$ ) and its targets *Penpla2* and *Lipe*, compared to WAT of wt; this was related to an increase in the amount of serum NEFA. Despite fasting hypoglycemia, Real Time-PCR experiments revealed increased levels of PEPCK, G6pase ( $p < 0.05$ ) and FoxO1 in IL-21 KO livers, compared to wt littermates livers. Western blot analysis showed reduced phosphorylation and acetylation of FoxO1 ( $p < 0.05$ ). RNA levels of inflammatory genes as F4/80 and CD68 were reduced in liver and WAT of IL-21 KO mice ( $p < 0.05$ ) compared to wt; this suggests a low infiltration of proinflammatory macrophages M1 in adipose tissue of IL21 KO mice. On the contrary, we found increased RNA expression of YM1 and Mgl2 ( $P < 0.05$ ) suggesting a high presence of alternatively activated macrophages M2.

**Conclusion:** Taken together, our data indicate that IL-21 KO mice are partially protected from the metabolic injury caused by HFD. Since IL21 KO mice fed a HFD showed tendency to hypoglycemia and increased lipolysis, further studies are needed to elucidate whether these effects are dependent from modulation on adipose tissue inflammation or are directly related to modulation of insulin sensitivity in both adipose and hepatic cells.

## 65

### Intestinal microbiota translocation is associated with inflamed visceral adipose tissue

M. Nieuwdorp<sup>1,2</sup>, A. Vrieze<sup>1</sup>, L. Jonker<sup>1</sup>, P. Surendran<sup>2</sup>, N. de Sonnaville<sup>1</sup>, G.M. Dallinga-Thie<sup>2</sup>, R.S. Kootte<sup>2</sup>, H.G.G. Heilig<sup>3</sup>, E.G. Zoetendal<sup>3</sup>, O.R.C. Busch<sup>4</sup>, S. van der Meij<sup>3</sup>, B. van Wagenveld<sup>6</sup>, W.M. de Vos<sup>3,7</sup>, F. Holleman<sup>1</sup>, J. Hoekstra<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands, <sup>2</sup>Department of Vascular Medicine, Academic Medical Center, Amsterdam, Netherlands, <sup>3</sup>Laboratory of Microbiology, Wageningen University, Netherlands, <sup>4</sup>Department of Surgery, Academic Medical Center, Amsterdam, Netherlands, <sup>5</sup>Department of Surgery, Flevo ziekenhuis, Almere, Netherlands, <sup>6</sup>Department of Surgery, Sint Lucas Andreas Ziekenhuis, Amsterdam, Netherlands, <sup>7</sup>Department of Basic Veterinary Medicine, University of Helsinki, Finland.

**Background and aims:** Inflammation of visceral fat is a key regulator in the development of insulin resistance and subsequent type 2 diabetes, yet the pathophysiological trigger for this chronic inflammation remains poorly understood. As the intestinal microbiota plays a pivotal role in metabolism, we hypothesized that altered faecal intestinal microbiota composition induces endotoxemia and subsequent visceral adipose tissue inflammation. Thus, the aim of our study was to investigate whether a) faecal intestinal microbiota differs between humans with and without inflamed adipose tissue and b) whether intestinal microbial DNA can be detected in adipose tissue.

**Material and methods:** Treatment naïve healthy men and women (Mean BMI 29.0 kg/m<sup>2</sup>), scheduled for elective laparoscopic cholecystectomy were included. Fasting metabolic markers and inflammation parameters (hsCRP and lipopolysaccharide-binding protein, LBP) were measured. At the day of surgery, patients collected stool samples for the deep and global phylogenetic profiling of the intestinal microbiota using the Human Intestinal Tract Chip (HITChip). During surgery visceral adipose tissue was obtained in sterile endotoxin free containers and immediately processed for macrophage (CD68) staining using immunohistochemistry and bacterial DNA detection.

**Results:** Based on plasma LBP we divided the patients into two groups: high ( $n=12$ ) vs. low LBP ( $n=12$ ) plasma levels. CD68 staining in the visceral adipose tissue showed LBP was positively associated with the accumulation of macrophages. Even though the number of adipocytes were similar between low and high LBP, CD68 staining revealed 48% higher number of macrophages in high LBP compared to the low LBP (8.5:4.1 per um of adipose tissue respectively,  $p < 0.0001$ ). In addition, the total group also showed significant correlation between plasma LBP and the amount of CLS, Spearman's  $\rho=0.4$ ,  $p < 0.05$ ). Finally, we demonstrated the presence of bacterial DNA in this visceral adipose tissue.

**Conclusion:** We show that plasma LBP is associated with macrophage accumulation in visceral fat tissue of otherwise healthy obese subjects. Since bacterial DNA was present in these visceral fat specimens, our data imply that intestinal microbiota may play an initiating role in chronic inflammation of visceral fat. Comparative analysis of the intestinal microbiota composition and diversity will be presented and discussed.

Clinical Trial Registration Number: NTR 2335

## 66

### Toll like receptor 3 initiates adipose tissue inflammation during obesity

J.A. van Diepen, E.J.P. van Asseldonk, D.B. Ballak, R. Stienstra, T.B. Koenen, H.J. Jansen, M.G. Netea, C.J. Tack; General Internal Medicine, UMC St Radboud Nijmegen, Netherlands.

**Background and aims:** Obesity is accompanied by a chronic low-grade inflammation that contributes to the development of insulin resistance and type 2 diabetes. In adipose tissue, obesity induces infiltration of macrophages and secretion of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6 and TNF $\alpha$ . Toll like receptors (TLRs) are the frontline of innate immunity and can sense specific exogenous and endogenous ligands. Both TLR2 and TLR4 have shown to play a role the development of obesity-associated adipose tissue inflammation. Although expression of TLR3 in adipocytes has been found, the functional effects of TLR3 in adipose tissue are unknown. In the present study we aimed to investigate the role of TLR3 in obesity-induced adipose tissue inflammation and insulin resistance.

**Materials and methods:** Human adipose tissue biopsies were used to determine the expression of TLR3 and its correlation with markers for obesity and adipose tissue inflammation. In addition, adipose tissue inflammation as well as insulin sensitivity were determined in TLR3<sup>-/-</sup> and Wildtype (WT) that were fed a high fat diet (HFD) or low fat diet (LFD) for 16 weeks.

**Results:** In a large series of human fat tissue samples ( $n=76$ ), expression of TLR3 was significantly correlated with BMI and expression of leptin ( $r^2 = 0.15$  and  $0.08$ ;  $P < 0.01$ ), as well as expression of the pro-inflammatory cytokines IL-1 and TNF $\alpha$  ( $r^2 = 0.15$  and  $0.28$ ;  $P < 0.001$ ). TLR3 expression was highest in the adipocyte fraction as compared to the stromal vascular fraction in both the subcutaneous adipose tissue ( $1.00 \pm 0.26$  vs  $0.16 \pm 0.02$ ;  $P < 0.05$ ) and the visceral adipose tissue ( $1.03 \pm 0.17$  vs  $0.22 \pm 0.04$ ;  $P < 0.05$ ), suggesting a role of TLR3 in adipose tissue functioning. In response to HFD, TLR3<sup>-/-</sup> and WT mice had a similar increase in total body weight, but TLR3<sup>-/-</sup> mice displayed a reduction in white adipose tissue mass ( $-14\%$ ;  $P < 0.05$ ). Compared to LFD, HFD-feeding significantly increased *ex vivo* IL-6 secretion of adipose tissue explants (per mg tissue) derived from WT, while HFD did not affect IL-6 secretion of explants from TLR3<sup>-/-</sup> mice. Additionally, FACS analysis on the stromal vascular fraction showed that TLR3 deficiency blunted the HFD-induced increase in macrophages (F4/80+) content of adipose tissue. Remarkably, despite this reduction in adipose tissue inflammation, TLR3 deficient mice were not protected from HFD-induced systemic insulin resistance.

**Conclusion:** Our results show that the TLR3 is highly expressed in human adipose tissue and correlates with BMI as well as markers of adipose tissue inflammation. Animal studies reveal that TLR3 deficiency affects adipose tissue mass and protects against HFD-induced inflammation. These changes, however, do not translate into a reduction in systemic insulin resistance. As TLR3 is predominantly expressed in adipocytes (as opposed to stromal immune cells), TLR3 may be particularly involved in the early initiation of the inflammatory cascade in response to adipose tissue expansion.

## OP 12 Dynamics of beta cell signal transduction

67

### Caveolin-1 mediates the internalisation of functional insulin receptors in beta cells

T. Albrecht, G.E. Lim, I.R. Nabi, J.D. Johnson;  
Cellular and Physiological Sciences, University of British Columbia,  
Vancouver, Canada.

**Background and aims:** Type 2 diabetes is associated with defects in insulin receptor signalling, which has also been shown to cause beta-cell death. Thus, it is of fundamental importance to understand the mechanisms by which insulin transmits its pro-survival signals in beta-cells. The internalization of the activated insulin receptor (InsR) complex is necessary to initiate insulin signalling. The mechanisms of InsR internalization in beta-cells remain unclear, although Caveolin-1 (CAV1) has been implicated in other cell types. Our aim was to determine the proteins and membrane nanodomains involved in InsR internalization. Following the internalization of InsR in living cells required the development of novel fluorescent InsR fusion proteins because previous studies used InsR proteins labelled at the tyrosine kinase domain or the insulin-binding domain, which would disrupt signalling.

**Materials and methods:** We labelled the InsR with monomeric fluorescent proteins in a non-critical linker region between the furin-like region and the transmembrane domain. TagBFP and TagRFP fusion proteins were generated for Rab5, Rab7, Rab11, markers of endosomal populations. CAV1:RFP and LAMP2:GFP were employed to investigate an endocytic pathway and lysosomal degradation, respectively. We monitored InsR trafficking in MIN6 beta-cells using deconvolution microscopy, confocal microscopy, and fast live cell total internal reflection microscopy (TIRFM). Super-resolution stimulated emission depletion (STED) microscopy was applied to resolve the organization of membrane nanodomains harbouring the insulin receptor.

**Results:** TagRFP labelled InsR were shown to be fully functional via the assessment of ERK1/2 phosphorylation after 50 nM insulin treatment. The functionality of our novel InsR:TagRFP fusion protein was further confirmed by its co-internalization with fluorescently labelled insulin. To determine if CAV1 is involved in InsR internalization in the beta-cell we co-expressed fluorescently labelled CAV1:mTFP and InsR:TagRFP. TIRFM in living cells revealed that CAV1 is recruited to InsR membrane domains right before receptor internalization. These results were confirmed by STED microscopy, which showed a CAV1 protein coat around membrane nanodomains containing InsR. To investigate if CAV1 is essential for InsR internalization we compared the intracellular distribution of InsR in cells expressing CAV1 mutant proteins and RNAi. Indeed, MIN6 cells over-expressing dominant negative CAV1-Y14F:RFP showed defects in InsR internalization, with virtually complete accumulation of InsR at the plasma membrane. Conversely, cells over-expressing the dominant positive CAV1-Y14D:RFP mutant had excess InsR internalization into large vesicles, also strongly suggesting a requirement for CAV1 in InsR internalization. Effects of CAV1 mutants on the activation of Erk and Akt phosphorylation by insulin were investigated using Western blot. InsR:TagRFP-positive endosome populations were rapidly trafficked to lysosomes by a pathway that largely bypassed the classical Rab5 or Rab7 routes.

**Conclusion:** Taking advantage of our novel and validated InsR:TagRFP construct, we were able to monitor the dynamics of fully functional InsR in living beta-cells for the first time. We identified CAV1 as a key protein required for InsR internalization and elucidated the route of InsR within beta-cells. Together, our data give new insights on the mechanisms in InsR signalling.

Supported by: JDRE, Karl-Heinz Frenzen-Stiftung

68

### Chelation of $Zn^{2+}$ interferes with phasic insulin release by relieving feedback inhibition

O. Dyachok, A. Tengholm, E. Gylfe;  
Department of Medical Cell Biology, Uppsala University, Sweden.

**Background and aims:** Insulin hexamers form crystals with two coordinating zinc atoms within the  $\beta$ -cell granules. During exocytosis the crystals rapidly dissolve, releasing insulin and free  $Zn^{2+}$ . We have previously reported that released  $Zn^{2+}$  re-enters MIN6  $\beta$ -cells through voltage-dependent  $Ca^{2+}$  channels and inhibits insulin secretion (IS) by lowering cAMP. The present

study was undertaken to clarify the autocrine effect of  $Zn^{2+}$  on the kinetics of IS from mouse pancreatic islets.

**Materials and methods:** Epifluorescence microfluorometry and Meso Scale immunoassay were used for parallel measurements of the cytoplasmic  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) and IS from groups of 3–5 superfused pancreatic islets loaded with the  $Ca^{2+}$  indicator Fura-2LR.  $Zn^{2+}$  feedback was prevented using the novel fast-acting extracellular  $Zn^{2+}$  chelator ZX-1 and the cell-permeable heavy metal chelator TPEN under conditions when IS from the islets were stimulated either with 20 mM glucose alone or with 30 mM KCl in the presence of 20 mM glucose and 250  $\mu$ M of the KATP channel activator diazoxide.

**Results:** Islets stimulated by rise of glucose from 3 to 20 mM reacted with initial reduction of  $[Ca^{2+}]_i$  followed by pronounced rise and fast oscillations from an elevated level. IS showed a distinct initial peak followed by a slowly rising second phase. Introduction of ZX-1 slightly reduced  $[Ca^{2+}]_i$ , converting the fast oscillations into very regular ones with increased frequency, and amplified secretion beyond the initial peak. These fast regular oscillations are reminiscent to those obtained with elevation of cAMP. When glucose was increased in the presence of ZX-1 the initial  $[Ca^{2+}]_i$  responses were followed by very regular fast  $[Ca^{2+}]_i$  oscillations. Under these condition both first and second phase secretion were pronounced and clearly separated. Omission of ZX-1 slightly raised  $[Ca^{2+}]_i$  and decreased frequency of the superimposed fast oscillation that also became less regular. To identify  $[Ca^{2+}]_i$ -independent effects of ZX-1 we performed experiments under conditions when  $[Ca^{2+}]_i$  is stably elevated by KCl depolarization.  $[Ca^{2+}]_i$  and IS were at basal levels in islets exposed to 20 mM glucose in the presence of diazoxide. Increase of the KCl concentration to 30 mM induced a prompt  $[Ca^{2+}]_i$  rise reaching a maximum in 25–40 sec and then settling at a somewhat lower constant elevation within 2 min. Parallel insulin measurements showed a similar pattern with an initial peak followed by sustained stimulation. Chelation of extracellular  $Zn^{2+}$  with 100  $\mu$ M ZX-1 caused a barely detectable reduction of the sustained  $[Ca^{2+}]_i$  level but IS was stimulated and even exceeded the initial peak. These effects were readily reversible and subsequent chelation of all  $Zn^{2+}$  with TPEN stimulated secretion without affecting  $[Ca^{2+}]_i$ . The  $[Ca^{2+}]_i$  pattern was almost identical when islets were depolarized with KCl in the presence of ZX-1 but IS increased to a sustained rate with a less distinct first peak. Subsequent omission of ZX-1 marginally raised  $[Ca^{2+}]_i$  but markedly reduced IS. The effects were reversed by reintroduction of ZX-1 and there was no additional effect of TPEN.

**Conclusion:** The present data show that trapping of  $Zn^{2+}$  co-released with insulin interferes with a negative feed-back loop that affects phasic release of the hormone. The findings are consistent with the concept that released  $Zn^{2+}$  re-enters the  $\beta$ -cell and inhibits secretion by lowering cAMP.

69

### Dynamic control of Epac2 localisation and Rap activity in glucose-stimulated beta cells

I. Jakobsson, O. Idevall-Hagren, A. Tengholm;  
Department of Medical Cell Biology, Uppsala, Sweden.

**Background and aims:** Glucose stimulation of insulin secretion involves elevation of the cytoplasmic  $Ca^{2+}$  and cAMP concentrations in pancreatic  $\beta$ -cells. The effects of cAMP are partly mediated by Epac2, which is a guanine nucleotide exchange factor for the Rap family of small GTPases. The aim of the present study was to investigate how glucose and cAMP affects the subcellular localization of Epac2 and the activity of Rap GTPases in the  $\beta$ -cell.

**Materials and methods:** Immunocytochemistry and confocal imaging was used to determine the subcellular localization of endogenous Epac2. Fluorescent protein-tagged Epac2 and evanescent wave microscopy enabled dynamic imaging of plasma membrane association of the protein in MIN6-cells and primary mouse  $\beta$ -cells. Epac2 mutants unable to interact with cAMP or Ras were generated by introducing point mutations in their binding domains. The localization of active Rap was visualized with a green fluorescent protein-tagged Rap-GTP binding protein domain (GFP-RaGDS<sub>RBD</sub>).

**Results:** In unstimulated cells exposed to 3 mM glucose Epac2 was distributed in the cytoplasm. Elevation of the glucose concentration to 11 or 20 mM triggered translocation of Epac2 to the plasma membrane in both MIN6 cells and primary mouse  $\beta$ -cells. The translocation was often cyclic with a periodicity of 2–8 minutes, reminiscent of the cAMP and  $Ca^{2+}$  oscillations observed in glucose-stimulated  $\beta$ -cells. Elevation of the intracellular cAMP concentration with 100  $\mu$ M of the phosphodiesterase inhibitor IBMX or by direct application of cAMP to cells permeabilized with  $\alpha$ -toxin also triggered plasma membrane translocation of Epac2. Mutation of the N-terminal, low-



affinity cAMP-binding site of Epac2 did not affect translocation. In contrast, substitution of a single amino acid residue in the high-affinity cAMP-binding site abolished the Epac2 translocation in response to cAMP. Similarly, a point mutation in the Ras association domain also prevented translocation of Epac2 in response to cAMP elevation. Epac2 translocation was associated with activation of Rap at the plasma membrane, since an increase of the glucose concentration from 3 to 11 or 20 mM or elevation of intracellular cAMP with IBMX induced membrane translocation of the Rap activity reporter GFP-RalGDS<sub>RBD</sub>. Glucose-induced Rap activity often showed distinct oscillations in both MIN6-cells and primary mouse  $\beta$ -cells.

**Conclusion:** Glucose stimulation of  $\beta$ -cells induces cyclic Epac2 translocation to the plasma membrane. The translocation requires intact high-affinity cAMP-binding- and Ras-association domains of the protein. The resulting cyclic activation of Rap activity at the plasma membrane is likely involved in the generation of pulsatile insulin secretion.

*Supported by: EFSD/MSD grant and the Novo-Nordisk Foundation*

## 70

### Physiological concentrations of GLP-1 increase insulin secretion by activating protein kinase C pathway in pancreatic beta cells

M. Shigeto<sup>1,2</sup>, R. Ramracheya<sup>1</sup>, N. Rorsman<sup>1</sup>, M. Katsura<sup>2</sup>, B. Thorens<sup>3</sup>, K. Kaku<sup>2</sup>, P. Rorsman<sup>1</sup>;

<sup>1</sup>University of Oxford, UK, <sup>2</sup>Department of Diabetes, Endocrinology and Metabolism, Kawasaki Medical School, Kurashiki, Japan, <sup>3</sup>Physiology, University of Lausanne, Switzerland.

**Background and aims:** The peripheral basal blood concentration of GLP-1 (Glucagon like peptide-1) is in the picomolar range. Moreover, administration of dipeptidyl peptidase (DPP)-IV inhibitors increase the peripheral blood concentration of GLP-1 by only a few picomolar and yet results in significant anti diabetic effects. However, most *in vitro* experiments studying the effect of GLP-1 routinely use nanomolar concentrations. We have previously reported that a low concentration (1 pM) of GLP-1 stimulated insulin secretion through a cAMP-PKA (cyclic AMP-protein kinase A) independent pathway in mouse  $\beta$ -cells. In this study, we tried to identify this signalling pathway by which the physiological concentrations of GLP-1 affect insulin secretion from primary  $\beta$ -cells. We now confirm that GLP-1 induced insulin secretion have PKC (protein kinase C) dependent pathway and L-type calcium channel is involved in the pathway.

**Materials and methods:** Mouse islets were isolated by collagenase digestion. Secreted insulin was quantified using the RIA kit. Dispersed cells were plated on plastic tissue culture dishes for electrophysiological recordings. Recordings were performed using the perforated-patch whole-cell configuration. Experiments of GLP-1 receptor knockout mice were performed in University of Lausanne. Data are presented as mean  $\pm$  SEM. Statistical significance was evaluated using the Student t-test.

**Results:** GLP-1 (1 pM) stimulated insulin secretion from perfused mouse pancreas (N=8) and isolated islets ( $16.3 \pm 1.6$  vs  $23.0 \pm 4.0$  pg/islet/h, N=7,  $p < 0.05$ ). GLP-1 also increased  $\beta$ -cell membrane capacitance ( $6.8 \pm 4.45$  vs  $35.8 \pm 17.7$  fF, N=5,  $p < 0.05$ ) and calcium current ( $I_{Ca}$ :  $2.61 \pm 0.46$  vs  $3.21 \pm 0.59$ , N=7,  $p < 0.05$ ) on single  $\beta$ -cell. The effect was completely inhibited by addition of GLP-1 receptor blocker, exendin9-39 (100 nM). Insulin secretion induced by 10 nM GLP-1 was inhibited by PKA inhibitor, RP-cAMPS (100  $\mu$ M), while 1 pM GLP-1 was still significantly increased insulin secretion ( $16.3 \pm 1.6$  vs  $22.6 \pm 4.3$  pg/islet/h, N=9,  $p < 0.05$ ) and  $I_{Ca}$  ( $1.93 \pm 0.19$  vs  $2.24 \pm 0.18$ , N=5,  $p < 0.05$ ). The PKC inhibitor, bisindolylmaleimide (100 nM) and calphostin C (100 nM), blocked the effect of 1 pM GLP-1 on insulin secretion and  $I_{Ca}$ . GLP-1 stimulated insulin secretion was significantly inhibited by L-type calcium channel blocker, isradipine (2  $\mu$ M). Surprisingly, GLP-1 further decreased calcium currents in the presence of isradipine ( $0.678 \pm 0.09$  vs  $0.59 \pm 0.09$ , N=5,  $p < 0.05$ ). Neither 1 pM nor 10 nM stimulated insulin secretion from islets of GLP-1 receptor knockout mice. One pM of GLP-1 increased insulin secretion ( $63.2 \pm 11.5$  vs  $121.1 \pm 23.2$  pg/islet/h, 2 donors,  $p < 0.05$ ) and  $I_{Ca}$  ( $3.66 \pm 1.14$  vs  $4.07 \pm 1.17$ , N=5,  $p < 0.05$ ) on human.

**Conclusion:** The results demonstrate that physiological concentrations of GLP-1 increases insulin secretion, and suggest that PKC and L-type calcium channel are involved in its signal pathway in mouse. It would appear that both PKA and PKC pathway are working with concerted efforts *in vivo*.

## 71

### Involvement of phosphodiesterases in the shaping of sub-membrane-cAMP signals and pulsatile insulin secretion

G. Tian, J. Sageterp, A. Tengholm;

Medical cell biology, Uppsala University, Sweden.

**Background and aims:** Cyclic AMP (cAMP) regulates numerous cell functions, including insulin secretion from pancreatic  $\beta$ -cells. Specificity and versatility of cAMP signalling are determined by the spatial localization and temporal dynamics of the signal. Phosphodiesterases (PDEs) are important terminators of cAMP signals by degrading the nucleotide. In  $\beta$ -cells, glucose triggers oscillations of the cAMP concentration beneath the plasma membrane ([cAMP]<sub>pm</sub>) which are important for kinetic control of insulin secretion, but the mechanisms underlying the oscillations are poorly understood. The aim of this study was to clarify the role of different PDEs for the generation of [cAMP]<sub>pm</sub> oscillations in glucose-stimulated  $\beta$ -cells.

**Materials and methods:** A translocation biosensor based on fluorescence-tagged subunits of protein kinase A was used to monitor [cAMP]<sub>pm</sub> in insulin-secreting MIN6 cells and mouse pancreatic  $\beta$ -cells. The time-course of insulin secretion from single MIN6 cells was monitored using a translocation sensor for PtdIns(3,4,5)P<sub>3</sub>, which is formed in the plasma membrane following autocrine insulin receptor activation. Changes in localization of the biosensors were detected with total internal reflection fluorescence microscopy. Pharmacological agents were used to achieve family-selective PDE inhibition, and lentiviral vectors expressing shRNA to knock-down PDE8B.

**Results:** [cAMP]<sub>pm</sub> was low and stable in  $\beta$ -cells exposed to basal medium containing 3 mM glucose. The general PDE inhibitor IBMX caused a prompt increase of [cAMP]<sub>pm</sub>. While oscillations were frequently observed at 50  $\mu$ M IBMX, 300  $\mu$ M of the inhibitor most often caused stable [cAMP]<sub>pm</sub> elevation. In both MIN6- and islet  $\beta$ -cells inhibition of transmembrane adenylyl cyclases with 100  $\mu$ M 2',5'-dideoxyadenosine (DDA) resulted in marked suppression of [cAMP]<sub>pm</sub> despite the presence of IBMX, indicating the involvement of IBMX-insensitive mechanisms in cAMP degradation. Among IBMX-sensitive PDEs, PDE3 was most important for maintaining a low [cAMP]<sub>pm</sub> under basal conditions in both the clonal and primary  $\beta$ -cells. After glucose induction of [cAMP]<sub>pm</sub> oscillations, inhibitors of PDE1, -3 and -4 increased the average [cAMP]<sub>pm</sub>, but typically did not prevent the oscillations in either type of  $\beta$ -cells. Gene silencing by shRNA was used to assess a role of the IBMX-insensitive isoform PDE8B in glucose-induced [cAMP]<sub>pm</sub> signaling. Knockdown of PDE8B expression in MIN6-cells resulted in increased basal [cAMP]<sub>pm</sub> and prevention of the [cAMP]<sub>pm</sub>-lowering effect of DDA after IBMX exposure. Furthermore, PDE8B knockdown perturbed the glucose-induced [cAMP]<sub>pm</sub> oscillations and increased insulin secretion with loss of the normal pulsatility.

**Conclusion:** [cAMP]<sub>pm</sub> oscillations in  $\beta$ -cells are caused by cyclic variations in the rate of cAMP production. Several PDEs, including the IBMX-insensitive PDE8B, are involved in shaping the sub-membrane cAMP signals and pulsatile insulin secretion.

*Supported by: EFSD/MSD grant and Novo Nordisk Foundation*

## 72

### The scaffold protein beta-arrestin 2 is involved in the modulation of pancreatic beta cell mass

M.A. Ravier<sup>1</sup>, N. Linck<sup>1</sup>, A. Varrault<sup>1</sup>, N. Piro<sup>2</sup>, A. Tamazouzt<sup>1</sup>, S. Dalle<sup>1</sup>, G. Bertrand<sup>1</sup>;

<sup>1</sup>Institut de Genomique Fonctionnelle, Montpellier, <sup>2</sup>Réseau d'Histologie Expérimentale de Montpellier, France.

**Background and aims:** The autocrine action of insulin on its own receptor was shown to be critical for the maintenance of normal pancreatic  $\beta$ -cell function and mass. It has been reported that downstream of the insulin receptor signalling, besides the conventional IRS/PI3K/Akt pathway, a second pathway involving  $\beta$ -arrestin 2 ( $\beta$ -arr2) is required to induce a full Akt activation in hepatocytes, and that any deficiency of this signal contributed to the development of insulin resistance. As we have observed similar mechanisms in pancreatic  $\beta$ -cells, the aim of our study was to investigate the functional role of  $\beta$ -arr2 in pancreatic  $\beta$ -cells using  $\beta$ -arr2 KO mice.

**Materials and methods:**  $\beta$ -arr2 KO mice and their wild-type (WT) littermates were fed with either a standard diet (5 kcal% fat) or a high fat diet (HFD, 45 kcal% fat) from weaning for 22-25 weeks. Islet size and areas were determined by morphometric analysis from sections of paraffin embedded pancreas stained with hematoxylin and eosin.  $\beta$ -cell mass and islet architec-

ture were assessed by immunofluorescence.  $\beta$ -arr2 expression was evaluated in isolated islet by quantitative real time-PCR and Western blotting.

**Results:** In  $\beta$ -arr2 KO mice, insulin secretion in response to glucose was not affected, while the insulin content of pancreas and islets, as well as the mRNA level of Ins2 in islets, were decreased by about 25% ( $p < 0.05$ ), suggesting a role for  $\beta$ -arr2 in the modulation of  $\beta$ -cell mass. Indeed, morphometric histological analysis of pancreatic sections showed a reduction of the total islet area (30%,  $p < 0.05$ ) in  $\beta$ -arr2 KO mice, due to a modified distribution of islet sizes with an increased proportion of small islets ( $< 0.002 \text{ mm}^2$ ,  $p < 0.01$ ) and a reduction of large islets ( $> 0.01 \text{ mm}^2$ ,  $p < 0.05$ ). On the other hand, immunohistochemical staining of insulin and glucagon did not show any abnormalities in islet architecture but, in keeping with reduced islet size, indicated a decrease in  $\beta$ -cell number (35%,  $p < 0.01$ ), and therefore  $\beta$ -cell mass. Furthermore,  $\beta$ -arr2 mRNA and protein expression were down regulated by 25% ( $p < 0.05$ ) in pancreatic islets of WT mice fed with a high fat diet (HFD). Remarkably, under HFD, while WT littermate mice displayed a doubling of their islet area,  $\beta$ -arr2 KO mice were unable to compensate their  $\beta$ -cell mass ( $p < 0.05$ ).

**Conclusion:** The scaffold protein  $\beta$ -arr2 plays a role in the maintenance of  $\beta$ -cell mass in normal conditions. A reduced expression of the protein in insulin resistant state might prevent the compensatory  $\beta$ -cell mass expansion occurring in type 2 diabetes.

## OP 13 Nutritional approaches to body composition and liver fat

73

### Pioglitazone versus berberine for treatment of non-alcoholic fatty liver disease patients with impaired glucose regulation or type 2 diabetes mellitus

H. Yan<sup>1</sup>, M. Xia<sup>1</sup>, X. Chang<sup>1</sup>, H. Bian<sup>1</sup>, Q. Xu<sup>1</sup>, X. Gao<sup>1</sup>, Y. Tu<sup>2</sup>, W. Jia<sup>3</sup>, W. Deng<sup>3</sup>;

<sup>1</sup>Endocrinology and Metabolism, Zhongshan Hospital, Fudan University,

<sup>2</sup>Endocrinology and Metabolism, The sixth people's hospital, Shanghai

Jiaotong University, <sup>3</sup>School of public health, Fudan University, Shanghai, China.

**Background and aims:** To assess the effect and safety of pioglitazone (PGZ) or berberine (BBR) on Non-Alcoholic Fatty Liver Disease (NAFLD) patients with impaired glucose regulation (IGR) or diabetes mellitus (DM).

**Materials and methods:** Totally 184 subjects were enrolled in a randomized, open, controlled clinical trial with a 16-week period of follow-up. All the participants were diagnosed as NAFLD and IGR or DM, and were divided into three groups randomly: Group A received lifestyle intervention (LI) without any drugs ( $n=62$ ); Group B received LI and PGZ (15 mg qd.) ( $n=60$ ); Group C received LI and BBR (0.5g tid) ( $n=62$ ). All of them were treated for 16 weeks. The primary outcome was an improvement of metabolism, including fasting glucose, 2 hour glucose, HbA1c, lipid profile [total cholesterol (TC), triglyceride (TG), HDL-c, LDL-c], liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyltransferase (GGT)]. The secondary outcome was a reduction in liver fat content detected by proton magnetic resonance spectroscopy (<sup>1</sup>H MRS).

**Results:** The three groups were well matched in demographic characteristics, clinic and most of laboratory data, liver fat content and other aspects. Compared with pre-treatment data, subjects had significant improvement in HbA1c, liver fat content, ALT, AST, GGT, weight, waist circumference, WHR, diastolic blood pressure (of group A,B,C), fasting glucose, 2-hour glucose, systolic blood pressure (of group B,C), HDL-c (only group B), TC, TG, LDL-c (only group C) after 16 weeks treatment (all paired  $t$ -test  $p < 0.05$ ). Group B (-41.02%) and Group C (-51.30%) had more obvious reduction in liver fat content (percentage decline relative to the baseline) than Group A (-27.98%) (ANOVA  $p=0.034$ ), and the difference between group C and group A was statistically significant (Dunnett test  $p=0.035$ ). In multiple comparisons, PGZ therapy had a significantly higher reduction than LI in 2-hour glucose, ALT (all  $p < 0.05$ ). While BBR therapy, compared with LI, had a significantly higher reduction in TC, TG, ALT, weight, waist circumference, diastolic blood pressure (all  $p < 0.05$ ). Although PGZ or BBR therapy had significantly higher reduction in FBG, HbA1c, HDL-c, LDL-c, AST, GGT, systolic blood pressure, WHR, compared with LI, the differences of the three groups were not statistically significant (all  $p > 0.05$ ). The total incidence of adverse reactions of PGZ and BBR were 23.33% and 43.55% respectively. The major side effects of PGZ were muscle pain (26.32%), palpitations (10.53%), fatigue (10.53%). The major side effects of BBR were digestive symptoms such as anorexia (30.95%), diarrhea (19.05%), severe constipation (11.90%).

**Conclusion:** For NAFLD patients with IGR or DM, lifestyle intervention with or without pioglitazone or berberine are effective methods to improve glucose metabolism, reduce liver fat content and liver enzymes. Berberine is superior to lifestyle intervention and pioglitazone for improvement of lipid profile, weight, waist circumference and blood pressure. Both pioglitazone and berberine are well tolerated.

Clinical Trial Registration Number: NCT00633282

Supported by: STCSM Innovation Action Plan

74

### The effect of frequency of meals on hepatic fat content in patients with type 2 diabetes

L. Belinova<sup>1</sup>, H. Kahleova<sup>1</sup>, M. Hajek<sup>2</sup>, M. Dezortova<sup>2</sup>, M. Hill<sup>3</sup>, T. Pelikanova<sup>1</sup>;

<sup>1</sup>Department of Diabetes, Institute for Clinical and Experimental Medicine,

<sup>2</sup>Radiodiagnostic and Interventional Radiology Department, Institute for

Clinical and Experimental Medicine, <sup>3</sup>Institute of Endocrinology, Prague, Czech Republic.

**Background and aims:** Increased hepatic fat content is associated with type 2 diabetes (T2D). Caloric restriction is crucial in its treatment typically appor-



tioned into five or six small meals during the day. The aim of our study was to compare the effect of six meals vs. two meals a day with the same caloric restriction on hepatic fat content in subjects with T2D.

**Materials and methods:** In a randomized, crossover study, we assigned 54 patients with T2D to follow two regimens of a hypocaloric diet (-500 kcal/day), each for 12 weeks: six meals a day (A), and two meals a day (B). The diet in both regimens had the same macronutrient and energy content. We measured the hepatic fat content by the proton magnetic resonance spectroscopy performed by 3T MR scanner (Magnetom - Trio Siemens). Spectra were obtained from three different parts of the liver. Signal intensities were used for determining fat content. Then the fat total signal area (FTSA) ratio was calculated. For statistical analysis, 2x2 crossover ANOVA and Pearson's correlations were used.

**Results:** FTSA was reduced in response to both regimens ( $p<0.001$ ), more in B (-3.4; 95% CI, -3.8 to -3.1 % in A vs. -4.2; 95% CI, -4.5 to -3.8 % in B;  $p=0.03$ ). Body-mass-index (BMI) was reduced in both regimens ( $p<0.001$ ), more in B (-0.82; 95% CI -0.94 to -0.69 kg.m<sup>-2</sup> in A vs. -1.23; 95% CI -1.4 to -1.17 kg.m<sup>-2</sup> in B;  $p<0.001$ ). Fasting glycemia decreased in both regimens ( $p<0.001$ ), more in B (-0.47; 95% CI -0.57 to -0.36 mmol/l in A vs. -0.78; 95% CI -0.89 to -0.68 mmol/l in B;  $p=0.004$ ). Decrease in FTSA correlated strongly positively with decrease in fasting glycemia ( $r=+0.56$ ;  $p<0.001$ ). After adjustment for changes in BMI this association remained significant ( $r=+0.28$ ;  $p=0.05$ ).

**Conclusion:** Prolonged fasting reduced hepatic fat content more than a diet with the same caloric restriction divided in more frequent meals in patients with T2D. This reduction was associated with a decrease in fasting glycemia, independently on changes in BMI.

Clinical Trial Registration Number: NCT01277471

Supported by: VZ MZO 00023001

## 75

### The effect of frequency of meals on body weight, HbA<sub>1c</sub> and resting energy expenditure in patients with type 2 diabetes

H. Kahleova<sup>1</sup>, L. Belinova<sup>1</sup>, M. Hill<sup>2</sup>, T. Pelikanova<sup>1</sup>;

<sup>1</sup>Diabetes Centre, Institute for Clinical and Experimental Medicine,

<sup>2</sup>Institute of Endocrinology, Prague, Czech Republic.

**Background and aims:** Caloric restriction is crucial in the treatment of type 2 diabetes (T2D), typically (but not necessarily) apportioned into five or six small meals during the day. It has been shown that a large isocaloric mixed meal causes a greater postprandial thermogenic response than the same food consumed in six smaller portions. The aim of our study was to compare the effect of six vs. two meals a day with the same caloric restriction on body weight, HbA<sub>1c</sub> and resting energy expenditure in subjects with T2D.

**Materials and methods:** In a randomised, crossover study, we assigned 54 patients with T2D to follow two regimens of a hypocaloric diet (-500 kcal/day), each for 12 weeks: six meals a day (A), and two meals a day (B). The diet in both regimens had the same macronutrient and energy content. At weeks 0, 12 and 24, we measured subject weight, HbA<sub>1c</sub> and performed indirect calorimetry using metabolic monitor VMAX; Sensor Medics, Anaheim, CA, USA. Predicted resting energy expenditure (REE) was counted according to Harris-Benedict equation. For statistical analysis, 2x2 crossover ANOVA was used.

**Results:** Body weight decreased in both regimens ( $p<0.001$ ), more in B (-2.3; 95% CI -2.7 to -2.0 kg in A vs. -3.7; 95% CI -4.1 to -3.4 kg in B;  $p<0.001$ ). HbA<sub>1c</sub> decreased in both regimens ( $p<0.001$ ) with a trend toward a greater decrease in B (-0.23; 95% CI -0.27 to -0.19 % in A vs. -0.25; 95% CI -0.29 to -0.20 % in B;  $p=0.08$ ). Fasting C-peptide decreased in both regimens ( $p<0.001$ ), more in B ( $p=0.05$ : -0.14; 95% CI -0.18 to -0.1 vs. -0.05; 95% CI -0.09 to -0.01 nmol/L in A). HbA<sub>1c</sub> decreased in both regimens ( $p<0.001$ ); the trend toward the greater decrease in B was not significant ( $p=0.08$ : -0.25; 95% CI -0.29 to -0.20 vs. -0.23; 95% CI -0.27 to -0.19 % in A). Plasma immunoreactive insulin, triglycerides and LDL-cholesterol decreased comparably in both regimens. No significant change in total or HDL-cholesterol was observed in either regimen. Respiratory quotient increased in both regimens ( $p<0.01$ ) with a trend toward a greater increase in A (+0.06; 95% CI +0.04 to +0.07 in A vs. +0.04; 95% CI +0.02 to +0.05 in B;  $p=0.2$ ). Measured REE decreased in both regimens ( $p<0.001$ ) with a trend toward a greater decrease in A (-108.3; 95% CI -125.3 to -91.5 kcal/day in A vs. -90.8; 95% CI -107.5 to -74.3 kcal/day in B;  $p=0.3$ ). If counted as percentage of the predicted REE according to Harris Benedict equation, REE decreased in both regimens ( $p<0.001$ ), with a trend toward a greater decrease in A (-4.6; 95% CI -5.6 to -3.7 % in A vs. -3.0; 95% CI -3.9 to -2.1 % in B;  $p=0.08$ ).

**Conclusion:** Two meals a day reduced body weight, fasting glycemia and plasma C-peptide more than a diet with the same caloric restriction divided

in six more frequent meals in patients with T2D. Our data suggest that fewer bigger meals may be better than smaller ones during the day for patients with T2D.

Clinical Trial Registration Number: NCT 01277471

Supported by: grant IGA MZCR NT/11238-4

## 76

### A MUFA diet reduces liver fat in type 2 diabetes patients in absence of weight loss and independently of postprandial lipaemia

L. Bozzetto<sup>1</sup>, G. Annuzzi<sup>1</sup>, L. Costagliola<sup>1</sup>, A. Prinster<sup>2</sup>, A. Giacco<sup>1</sup>,

A. Mangione<sup>1</sup>, P. Cipriano<sup>1</sup>, L. Patti<sup>1</sup>, C. Vigorito<sup>3</sup>, G. Riccardi<sup>1</sup>, A.A. Rivellese<sup>1</sup>;

<sup>1</sup>Department of Clinical and Experimental Medicine, Federico II University,

<sup>2</sup>Institute of Biostructure and Bioimaging, National Research Council,

<sup>3</sup>Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Federico II University, Naples, Italy.

**Background and aims:** Liver steatosis is associated with higher risk of type 2 diabetes (T2DM) and postprandial lipid abnormalities. No studies have evaluated the effects of isocaloric lifestyle interventions on hepatic fat content and postprandial lipid metabolism. Therefore the aims of the present study were to evaluate the effects of two generally recommended diets with/without an aerobic exercise training on liver fat content and postprandial lipaemia in the absence of weight loss in T2DM.

**Materials and methods:** Forty-five T2DM patients in satisfactory blood glucose control (HbA<sub>1c</sub> 6.6±0.8%), aged 35-70 years, were randomized to an 8-week treatment with: high carbohydrate/high fibre/low glycemic index diet (CHO/fibre group), or high MUFA diet (MUFA group), or high carbohydrate/high fibre/low glycemic index diet plus physical activity program (CHO/fibre+Ex group), or high MUFA diet plus physical activity program (MUFA+Ex group). Physical activity program consisted in two 45 min sessions per week of supervised light/medium intensity exercise. Before and after intervention hepatic fat content was measured by proton magnetic resonance spectroscopy. Triglyceride-rich lipoproteins (TRL), separated by preparative sequential ultracentrifugation, were evaluated at fasting and over six hours after a test meal of similar composition to the run-in diet at baseline and to the assigned diet at the end of the intervention.

**Results:** Dietary compliance was optimal and body weight remained stable during the intervention in all groups. Liver fat decreased significantly more in MUFA (-29%) and MUFA+Ex (-25%) than in CHO/fibre (-4%) and CHO/fibre+Ex groups (-6%) ( $p=0.006$ ). TRL triglyceride postprandial incremental AUC decreased in CHO/fibre (-36%) and increased in CHO/fibre+Ex (+9%), MUFA (+29%) and MUFA+Ex (+54%) ( $p=0.004$ ).

**Conclusion:** A MUFA rich diet induced a clinically relevant reduction in hepatic fat in T2DM in the absence of body weight loss and independently of a light/medium intensity supervised exercise program. This MUFA effect was not related to an improvement in postprandial lipaemia.

Clinical Trial Registration Number: NCT01025856

## 77

### Effects of $\omega$ 3 and $\omega$ 6 polyunsaturated fatty acids supplementation on plasma circulating metabolic markers and adipose tissue oxidative stress in fructose-fed rats

K. Louchami<sup>1</sup>, Z. Mellouk<sup>2</sup>, W. Malaisse<sup>1</sup>, A. Sener<sup>1</sup>, D. Ait Yahia<sup>2</sup>;

<sup>1</sup>Laboratory of Experimental Hormonology, Free University of Brussels,

Belgium, <sup>2</sup>Department of Biology, Es-Sénia University, Oran, Algeria.

**Background and aims:** High intake of dietary fructose has been shown to exert a number of adverse metabolic effects in humans and experimental animals.  $\omega$ 3 and  $\omega$ 6 polyunsaturated fatty acids have been the subject of extensive investigation regarding their possible benefit effects on components of the metabolic syndrome. The aim of the present study was to examine whether  $\omega$ 3 or  $\omega$ 6 polyunsaturated fatty acids supplementation improves insulin resistance, plasma circulating metabolic markers as well as oxidative stress in adipose tissue in high-fructose-fed rats.

**Material and methods:** Twenty four female Wistar rats were exposed to diets containing either 64% (w/w) starch and 5% sunflower oil (Ssun), or 64% fructose and either 5% sunflower oil (Fsun), 3.4% sunflower oil and 1.6% salmon oil (Fsal), or 3.4% sunflower oil and 1.6% safflower oil (Fsal) for 8 weeks. Blood pressure and plasma glucose, insulin, triglycerides and leptin were determined. Moreover, hepatic glucokinase and glycogen and adipose tissue lipid (TBARS, hydroperoxides) and protein (carbonyls) oxidation, ni-

tric oxide (NO) as well as antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GSH-Red) were measured. **Results:** After 2 months of experiment, Fsun rats exhibited higher blood pressure and plasma triglycerides, leptin and HbA1C as compared to Ssun rats. Plasma glucose, insulin, insulinogenic index and HOMA-IR were similar between these two groups. Liver glucokinase activity and glycogen levels were significantly lower in Fsun than in Ssun. In addition, administration of fructose to rats led to high adipose tissue TBARS, hydroperoxides, carbonyls and low NO levels relative to Ssun animals. Adipose tissue SOD, CAT and GSH-Red activities were lower in Fsun rats than in Ssun rats. Treatment of fructose rats with  $\omega 3$  or  $\omega 6$  reduced significantly blood pressure, triglycerides, leptin and HbA1C but little affected plasma insulin and insulinogenic index as compared to Fsun. Hepatic glucokinase and glycogen were greater only in Fsal group as compared to Fsun. Moreover, a decrease in TBARS, hydroperoxides, and carbonyls were observed with  $\omega 3$  and  $\omega 6$  supplementation. Feeding Fsal diet enhanced NO contents in adipose tissue. Additionally, SOD and GSH-Red activities were greater in Fsal and Fsaf groups, while CAT was higher only in Fsal group as compared to Fsun group.

**Conclusion:** These results suggest that dietary supplementation with  $\omega 3$  and  $\omega 6$  polyunsaturated fatty acids to fructose rats alleviated the effects of fructose by lowering blood pressure, HbA1C, triglyceride and leptin levels. On the other hand,  $\omega 3$  and  $\omega 6$  consumption improved the oxidative stress by decreasing lipid and protein oxidation and enhancing antioxidant enzymes. Hence  $\omega 3$  and  $\omega 6$  can be used as nutraceutical nutrients for the prevention and/or management of metabolic syndrome and disorders related to it.

## 78

### Overfeeding with polyunsaturated fat causes distinct effects on liver and body fat accumulation compared with saturated fat: a randomised controlled trial

U. Risérus<sup>1</sup>, F. Rosqvist<sup>1</sup>, D. Iggman<sup>1</sup>, J. Kullberg<sup>1</sup>, L. Johansson<sup>1</sup>, H.-E. Johansson<sup>1</sup>, I. Dahlman<sup>2</sup>, P. Arner<sup>2</sup>, H. Ahlström<sup>3</sup>;

<sup>1</sup>Public health and caring sciences, Uppsala University, <sup>2</sup>Medicine, Karolinska Institutet, Stockholm, <sup>3</sup>Uppsala University, Sweden.

**Background and aims:** We have recently shown that n-6 PUFA reduces liver fat content and improve metabolic disorders compared with saturated fats under weight stable conditions. It is however unclear whether dietary fat composition could determine the body deposition and distribution during moderate weight gain. We aimed to investigate the effects on body composition during overfeeding with either saturated or n-6 polyunsaturated fat.

**Materials and methods:** Thirty-nine lean (mean BMI: 20.7±2.1), young (mean age: 27±4) men and women were overfed for 7 weeks by adding muffins to their regular diet. Subjects were randomized in a double-blind manner to either consume muffins high in saturated fatty acids (SFA-diet; palm oil) or n-6 polyunsaturated fatty acids (PUFA-diet; sunflower oil). Apart from fatty acid composition, the content of fibre, total fat, carbohydrate and protein were identical in all muffins. The amount of muffins consumed was individually adjusted with a targeted weight gain of 3%. Body composition including liver fat, visceral and subcutaneous abdominal adipose tissue (VAT and SAT, respectively) total adipose tissue (TAT) and lean tissue was measured with and magnetic resonance imaging (MRI).

**Results:** There were no differences between groups at baseline in liver fat, VAT, SAT, TAT or lean tissue (all P>0.1). Both groups consumed a similar amount of additional energy (+750 kcal/day) and gained similarly in body weight (1.6±0.9 kg, p=0.94 for difference between groups) representing a mean percentage weight gain of ~2.6%. The subjects on the SFA-diet however had a ~6-fold higher increase in liver fat, and a ~2-fold higher increase in VAT and TAT compared with the subjects on the PUFA-diet (all P<0.05 for diff between groups). In contrast, the PUFA-diet had a two-fold higher increase in lean tissue (p<0.05; 0.86±0.62 litres and 0.31±0.68 litres, respectively).

**Conclusion:** During overfeeding, excess calories from n-6 PUFA causes less liver and abdominal fat deposition and more lean tissue gain than excess calories from saturated fat. This is the first evidence in humans to suggest distinct effects of dietary fatty acids on body composition during weight gain. The implications of these findings for the prevention of obesity and type 2 diabetes in the population warrants further investigation.

Clinical Trial Registration Number: NCT01038102

Supported by: The Swedish Research Council and Swedish Society of Medicine

## OP 14 Can we improve outcomes in diabetic pregnancy?

### 79

#### Atlantic DIP: what does selective screening for gestational diabetes miss?

G.M. Healy<sup>1</sup>, A. Vellinga<sup>2</sup>, L. Carmody<sup>1</sup>, G. Avalos<sup>2</sup>, E. Mustafa<sup>1</sup>, S. Khalil<sup>1</sup>, E. Noctor<sup>1</sup>, B. Kirwan<sup>1</sup>, F.P. Dunne<sup>1,2</sup>;

<sup>1</sup>Department of Endocrinology, University College Hospital,

<sup>2</sup>School of Medicine, National University of Ireland, Galway, Ireland.

**Background and aims:** Gestational diabetes mellitus (GDM) is the most common medical complication of pregnancy, with prevalence ranging 4–16% internationally. It leads to adverse foetal and maternal outcomes, which large studies have shown can be significantly reduced with treatment. There is debate about whether universal or risk-factor based screening is most appropriate but the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommends universal screening in populations with a high population prevalence of Type 2 diabetes. The ATLANTIC Diabetes in Pregnancy (DIP) group studies GDM across the population of the Atlantic coast of Ireland. The aim of this study was to compare pregnancy outcomes for universal screening (US) compared to selective screening (SS) for GDM based on Irish guidelines.

**Materials and methods:** During 2007–2008, US was employed and during 2009–2010 a SS approach was used, which included women on the basis of body mass index, age, family history, pregnancy history and ethnicity. Both strategies utilised a 2 hour 75g Oral Glucose Tolerance Test (OGTT) using IADPSG cut off values. The population was categorised into three groups: Normal Glucose Tolerance from the universal screening (NGT - US), GDM from universal screening (US-GDM) and GDM from selective screening (SS-GDM). The pregnancy outcomes according to screening strategy were compared.

**Results:** During US 5286 women were screened, 557 (10.5%) were diagnosed with GDM. During SS, 562 were screened and 465 were identified as having GDM. In view of the SS criteria, women who underwent SS were older and had a greater BMI at screening point (P<0.01). However more women in the in SS received their diagnosis after > 28 weeks of gestation (45.7% Vs 32.9 % P<0.001). The percentages of adverse events detected were lowest in the US - NGT group, higher in the SS- GDM group and highest in the US- GDM group (Table 1). If we assume US is the gold standard and detects the population prevalence for adverse outcomes in GDM pregnancies, the results of this study show that SS potentially misses 2.1% of GDM associated pre-eclampsia, 7.3% GDM associated adverse birth outcomes and 6.9% of the GDM associated neonatal admissions.

**Conclusion:** SS does not identify all women with GDM and thus, cases of pre-eclampsia, neonatal morbidities and neonatal unit admissions are not being correctly assigned to GDM. These women and newborns miss the opportunity for timely diagnosis and appropriate evidence based interventions which may limit adverse pregnancy outcomes and influence future health of the mother and her offspring.

Table 1: Comparison of outcomes in the three groups

	US-NGT (%)	SS-GDM (%)	US-GDM (%)	X <sup>2</sup> Trend
Pre-eclampsia	180 (4.0)	24 (5.4)	41 (7.5)	0.00
Macrosomia	158 (3.4)	21 (4.6)	27 (4.9)	0.04
LGA	763 (16.3)	89 (19.5)	121 (21.8)	0.01
Adverse Birth outcomes	694 (14.7)	112 (24.1)	175 (31.4)	0.00
NNU	324 (6.9)	70 (15.8)	123 (22.7)	0.00

Supported by: Health Research Board of Ireland

## 80

### Kidney dysfunction in adult offspring exposed in utero to type 1 diabetes is associated with alterations in genome-wide DNA methylation profile

J.-F. Gautier<sup>1</sup>, R. Porcher<sup>2</sup>, L.S. Fetita<sup>1</sup>, C. Abi Khalil<sup>3</sup>, F. Travert<sup>3</sup>, S.P. Choukem<sup>1</sup>, J.-P. Riveline<sup>4</sup>, S. Hadjadj<sup>5</sup>, E. Larger<sup>6</sup>, P. Boudou<sup>7</sup>, B. Blondeau<sup>8</sup>, R. Roussel<sup>9</sup>, E. Ravussin<sup>9</sup>, P. Vexiau<sup>1</sup>, M. Marre<sup>3</sup>;

<sup>1</sup>Department of Diabetes and Endocrinology, <sup>2</sup>Department of Biostatistics and Medical Computing, Saint-Louis University Hospital, University Paris-Diderot Paris-7, Paris, France, <sup>3</sup>Department of Diabetes and Endocrinology, Bichat University Hospital, University Paris-Diderot Paris-7, Paris, France, <sup>4</sup>Department of Diabetes and Endocrinology, Centre Hospitalier Sud Francilien, Corbeil-Essonnes, France, <sup>5</sup>Department of Diabetes and Endocrinology, Centre Hospitalier Universitaire, Poitiers, France, <sup>6</sup>Department of Diabetes and Endocrinology, Hotel Dieu Hospital, <sup>7</sup>Unit of Transfer in Molecular Oncology and Hormonology, Saint-Louis University Hospital, University Paris-Diderot Paris-7, Paris, France, <sup>8</sup>INSERM U872, Centre de Recherche des Cordeliers, <sup>9</sup>Physiology, Pennington Biomedical Research Center, Baton Rouge, USA.

**Background and aims:** We previously reported that foetal exposure to maternal Type 1 Diabetes (T1D) is associated with kidney dysfunction at adult age, thereby conferring a potential risk for high blood pressure and renovascular diseases. The aim of the present work was to search for potential epigenetic modifications related to these anomalies.

**Materials and methods:** A total of 29 adult, non diabetic offspring of T1D mothers (case group) was compared with 29 non-diabetic offspring of T1D fathers (control group) for the methylation profile of their leukocyte DNAs (27,578 CpG sites, Human Methylation 27 BeadChip, Illumina Infinium®). A subset of 19 cases and 18 controls were studied for baseline and amino acid-stimulated Glomerular Filtration Rate (GFR), and Effective Renal Plasma Flow (ERPF), using a <sup>51</sup>Cr EDTA plus <sup>125</sup>I-hippurate primed constant infusion technique.

**Results:** Case and control groups were well matched for age (26±6 vs 26±6 yr), sex ratio (55 vs 52% women), fasting plasma glucose (4.5±0.4 vs 4.5±0.3 mM), body mass index (median 22.6 (range 17.9–29.9) vs 22.4 (19–27.7) kg/m<sup>2</sup>), and birth weight (3200 (2750–4270) vs 3300 (2700–4540) g). A total of 214 CpG sites were methylated differently in cases and in controls (p value set at ≤0.01). Global methylation of these 214 sites was 5% lower (95%CI 3.8 to 6.9 %, p<0.0001) in the case vs control groups with 190 sites less methylated and 24 sites more methylated in the case vs control group. The relationship between global methylation of these 214 sites and baseline GFR was significantly different between groups (p<0.05), with a positive correlation in the case group (r = 0.50, p = 0.036, i.e., the more methylation, the higher the baseline GFR) but not in the control group (r = -0.23, p = 0.37). A similar pattern of correlation was observed for amino acid-stimulated ERPF. The gene encoding DNA methyltransferase 1 (DNMT1), a key enzyme in maintaining methylation patterns during cell division, was one of the genes less methylated in exposed subjects.

**Conclusion:** Differences in methylation profile may impact kidney function of adult offspring of T1D mothers. However, these differences may act through pathways not specific to kidney development, or function.

*Supported by: DRCD, INSERM and SFD*

## 81

### Transcriptomic analysis demonstrated a placental dysfunction during maternal diabetes

T. Bouckenooghe<sup>1</sup>, P. Perimenis<sup>1</sup>, E. Eury<sup>2</sup>, S. Lobbens<sup>2</sup>, G. Sisino<sup>1</sup>, P. Gosset<sup>3</sup>, J. Delplanque<sup>2</sup>, P. Fontaine<sup>4</sup>, L. Storme<sup>1</sup>, P. Froguel<sup>2</sup>, A. Vambergue<sup>1</sup>;

<sup>1</sup>EA4489, CHRU de Lille/Université Lille Nord de France, <sup>2</sup>UMR8199, Institut Pasteur de Lille/Université Lille Nord de France, <sup>3</sup>GHICL, Lille, France, <sup>4</sup>CHRU de Lille, France.

Nowadays, there is increasing evidence for a role of the perinatal environment in the metabolic programming of adult life. A disturbed intra uterine milieu, like maternal diabetes, can favor the occurrence of chronic diseases in adulthood. In animal models of streptozotocin (STZ) induced diabetes, few studies have assessed the potential role of the placenta and particularly the implication of feto-placental genes on fetal programming. In our work, we: 1) evaluate the consequences of maternal hyperglycemia on pups metabolism; 2) use systems biology approach to analyze differentially expressed placental genes to identify pathways involved in Intra Uterine Growth Retardation (IUGR) and in placental hypertrophy. We used 3 groups of animals:

DS (n=5, receiving 65 mg/kg of STZ at G7), D30 (n=9, receiving sequentially STZ & 75 mg/kg of Nicotinamide at G7) and a control group (n=9). We have evaluated metabolic parameters in mothers during gestation and in pups at birth. Placental whole genome expression was performed to identify genes differentially expressed between experimental groups (Illumina and qPCR for confirmation). Diabetes, in DS group is more pronounced than in D30 group. We have observed in our treated groups, an IUGR with placental hypertrophy. Histological observations showed a hypovascularization associated with an increase number of glycogen cells. These observations have been correlated with our transcriptomic analyses showing a modification of genes implicated in angiogenic and glycogen pathways. Especially, prolactin gene (Fold change>4) and protein were highly upregulated in the DS group partially explaining the hypovascularization that can be due to prolactin anti-angiogenic effect. To confirm this observation in human, we used placental samples collected from a cohort of patients with type 1 diabetes (cohort DIAMANT). As previously shown in our rat models, we observed an important increase in the placental expression of prolactin at mRNA (Fold change>18) and protein level in diabetic women. The two different models used in this study have shown an IUGR with placental hypertrophy. Maternal diabetes induces a profound modification of placental genes leading to a defect in angiogenesis. IUGR observed is the result of the placental hypovascularization and could profoundly modify the metabolic imprinting of the fetus.

*Supported by: DIAMENORD, Sanofi-Aventis, Lilly*

## 82

### Lower levels of vitamin D at first trimester of pregnancy are associated with higher risk of developing gestational diabetes mellitus

M. Lacroix, M.-C. Battista, M. Doyon, M.-F. Hivert, P. Perron; Physiology/endocrinology, Sherbrooke's University, Canada.

**Background and aims:** Gestational diabetes mellitus (GDM) results from an imbalance between insulin resistance and insulin secretion capacity during pregnancy. Vitamin D (25OHD) is suspected to be implicated in mechanisms of insulin regulation. A few reports identified low 25OHD (<75 nmol/L) as a potential risk factor for the development of GDM. However, data are scarce and inconsistent, potentially because of uneven adjustment for confounding factors. We hypothesized that lower 25OHD levels at 1<sup>st</sup> trimester are associated with higher risk of developing GDM during pregnancy, after taking into account potential confounders.

**Materials and methods:** We recruited and followed prospectively a cohort of pregnant women representative of the general population delivering at our institution. At 1<sup>st</sup> trimester (between 6–13 weeks), we performed anthropometric measurements and blood samples. Women were excluded if they had diabetes prior pregnancy or diagnosed at 1<sup>st</sup> trimester. Levels of 25OHD were measured by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). At 2<sup>nd</sup> trimester, diagnosis of GDM was made based on a standard 75g oral glucose tolerance test, following International Diabetes Federation criteria.

**Results:** This analysis included 558 participants: 512 women with normal glucose tolerance (NGT) and 46 women who developed GDM (incidence rate of GDM = 8%). Mean level of 25OHD in our cohort was 62.0 ± 19.2 nmol/L. At 1<sup>st</sup> trimester, participants who developed GDM were older (NGT = 28.2 ± 4.3 years old; GDM = 30.5 ± 4.9 years old; p = 0.001), but had similar mean body mass index (BMI) (NGT = 25.1 ± 5.4 kg/m<sup>2</sup>; GDM = 27.3 ± 7.0 kg/m<sup>2</sup>; p = 0.06) and body fat percentage (BFP) (NGT = 31.0 ± 8.2 %; GDM = 33.9 ± 10.8 %; p = 0.08) compared to NGT participants. In univariate comparison analysis, 25OHD levels at 1<sup>st</sup> trimester tended to be higher in NGT women than in GDM women (NGT = 62.4 ± 19.4 nmol/L; GDM = 57.1 ± 16.6 nmol/L) but the difference was not statistically significant (p = 0.07). This trend was confirmed in simple logistic regression model predicting GDM incidence (OR = 1.30 per decrease of one SD in 25OHD levels; p = 0.09). However, after adjustment for major potential confounding factors (age, season of blood sampling and vitamin D supplement intake), lower 25OHD levels were significantly associated with higher risk of developing GDM (OR = 1.50 per decrease of one SD in 25OHD levels; p = 0.03). In addition, this association remained statistically significant after further adjustment for BMI (OR = 1.44; p = 0.046) or for BFP (OR = 1.43; p = 0.049).

**Conclusion:** Our results suggest that lower 25OHD levels at 1<sup>st</sup> trimester are associated with increased risk of developing GDM, after taking into consideration important confounding factors such as age, season of blood sampling, vitamin D supplementation and adiposity measurements, in a population-based cohort.

*Supported by: Diabète Québec*



## 83

**The effect of real-time continuous glucose monitoring in diabetic pregnancy - a randomised controlled trial**A.L. Secher<sup>1,2</sup>, L. Ringholm<sup>1</sup>, H.U. Andersen<sup>3</sup>, P. Damm<sup>2,4</sup>, E.R. Mathiesen<sup>1,2</sup>;<sup>1</sup>Center for Pregnant Women with Diabetes, Department of Endocrinology, Rigshospitalet, <sup>2</sup>Faculty of Health Sciences, University of Copenhagen,<sup>3</sup>Steno Diabetes Center, <sup>4</sup>Center for Pregnant Women with Diabetes, Department of Obstetrics, Rigshospitalet, Copenhagen, Denmark.

**Background and aims:** Strict glycaemic control is the outermost crucial factor in reducing pregnancy complications in women with diabetes, but the effect of real-time continuous glucose monitoring (rt-CGM) in this population has not been thoroughly investigated before. The aim of this trial was to assess whether rt-CGM improves glycaemic control and pregnancy outcome in unselected women with pregestational diabetes.

**Materials and methods:** 123 women with type 1 diabetes and 31 women with type 2 diabetes were randomized to either intermittent use of rt-CGM for periods of up to six days at 8, 12, 21, 27 and 33 gestational weeks, in addition to routine care, or routine care only. HbA<sub>1c</sub>, self-monitored plasma glucose (SMPG), and severe hypoglycaemic events were registered. To optimize glycaemic control, rt-CGM data was evaluated with a diabetes caregiver. Pregnancy complications and outcomes were recorded, with macrosomia, defined as large for gestational age, as the primary outcome.

**Results:** Women assigned to rt-CGM (n=79) had similar duration of diabetes (median 10 (range 1-37) vs. 12 (1-38) years, p=0.38) and HbA<sub>1c</sub> at baseline (6.6 (5.3-10.0) vs. 6.8 (5.3-10.7) %, p=0.67) [49 (34-86) vs. 51 (34-93) mmol/mol] as women in the control arm (n=75). Rt-CGM was performed at least three times in pregnancy by 75% of the women. At 33 gestational weeks, HbA<sub>1c</sub> (6.1 (5.1-7.8) vs. 6.1 (4.8-8.2) %, p=0.40) [43 (32-62) vs. 43 (29-66) mmol/mol] and SMPG (6.2 (4.7-7.9) vs. 6.2 (4.9-7.9) mmol/l, p=0.64) were comparable regardless of rt-CGM use or not, and a similar fraction of women experienced severe hypoglycaemia (13 vs. 16%, p=0.55). The rates of macrosomia (43 vs. 30%, p=0.09) and other maternal-fetal outcomes were comparable between the groups.

**Conclusion:** In this randomized trial, intermittent use of rt-CGM in pregnancy did not improve glycaemic control or pregnancy outcome in women with pregestational diabetes.

*Clinical Trial Registration Number: NCT00994357*

*Supported by: EFSD/LifeScan grant*

## 84

**Pregnancy induced hypertensive disorder is related to HbA<sub>1c</sub>, BMI and weight gain in women with gestational diabetes**B. Barquiel Alcalá<sup>1</sup>, L. Herranz de la Morena<sup>1</sup>, I. Castro Dufoyny<sup>1</sup>, M.G. Llaro Casas<sup>1</sup>, P. Parra Ramírez<sup>1</sup>, M. Jáñez Furio<sup>2</sup>, L.F. Pallardo Sánchez<sup>1</sup>;<sup>1</sup>Diabetes Department, <sup>2</sup>Obstetrics Department, Hospital Universitario La Paz, Madrid, Spain.

**Background and aims:** Hypertensive disorder is linked to both gestational diabetes (GDM) and maternal pregestational overweight or obesity. Whether the severity of GDM seems to be related to this outcome, the influence of the glycaemic and weight evolution of the mother until the end of pregnancy is unknown. The aim of this study is to assess the relative influence of baseline carbohydrate intolerance, maternal pregestational weight and their changes on pregnancy hypertensive disorder in GDM women.

**Materials and methods:** 2568 GDM women were followed during the third pregnancy quarter in the Diabetes and Pregnancy Department from 1987 through 2008. Fasting glycaemia (FG) and area under the GDM diagnostic curve (AUC) (NDDG criteria) were considered as baseline glucose intolerance. Glycated haemoglobin A1c was monthly obtained as a glycaemic control parameter. Prepregnancy BMI (pre-BMI) was calculated on the basis of self-reported weight and the measured height. Weight gain ratio was defined as the quotient between total weight gain and the mean weight gain as suggested by the Institute of Medicine Guidelines for each woman's category of BMI. Univariate and adjusted multiple regression models were obtained. A p value under 0.05 was considered as statistical significance.

**Results:** The women's mean age was 32.9 ± 4.4 years old; 59 (2.5%) had chronic hypertension; median parity was 1 (IQ range 0-1); 458 (27.5%) were smokers in the group with the data available. Mean fasting glucose of the OGTT was 90.5 ± 14.6 mg/dl; mean HbA<sub>1c</sub> was 5.1 ± 0.4 %; mean prepregnancy BMI 24.75 ± 4.67 kg/m<sup>2</sup> and their median ratio of weight gain was 0.8 (IQ range 0.60-1.07). 175 (6.8%) women were diagnosed with gestational hypertension

(6.1 %) and/ or preeclampsia (0.7%). Univariate analysis showed an association of the hypertensive disorder with all the glycaemic and weight related parameters considered except for the AUC. Multivariate analysis showed that HbA<sub>1c</sub>, pre-BMI and weight gain ratio were independent predictors of the subsequent development of pregnancy hypertensive disorder when the data were controlled for age, chronic arterial hypertension, parity and urinary tract infection. The HbA<sub>1c</sub> excess risk [OR (95% CI)] was 1.69 (1.08-2.65) per unit increase, 1.19 (1.15-1.23) per BMI unitary increment and 1.58 (1.06-2.34) per increment of weight gain ratio. Attributable risk fractions were 40.8 (7.4-62.3)%, 16.0 (13.0-18.7)% and 36.7 (5.7-57.3)% respectively. The influence of glycaemic control and weight gain were enhanced after adjustment by tabaquism in a subgroup (n = 1664).

**Conclusion:** Gestational hypertensive disorder is predicted by evolutive HbA<sub>1c</sub>, which is a stronger predictor than OGTT FG or AUC. Excessive weight gain during pregnancy exerts an at least additive influence to pre-BMI. Indeed, gestational hypertensive disorders are independently related to HbA<sub>1c</sub>, BMI and a higher than recommended weight gain in women with gestational diabetes.

## OP 15 Role of the immune system in type 1 diabetes

85

### AntiGAD positivity: its prevalence and clinical impact in a large non-diabetic population: results from the HUNT study

E.P. Sørgjerd<sup>1</sup>, K. Midtthjell<sup>2</sup>, V. Grill<sup>1</sup>;

<sup>1</sup>Norwegian University of Science and Technology, Institute of cancer research and molecular medicine, Trondheim, <sup>2</sup>Norwegian University of Science and Technology, HUNT, Dept. of Public Health and general Practice, Levanger, Norway.

**Background and aims:** Development of autoimmune diabetes is strongly associated with autoantibody markers with high affinity towards the islet of Langerhans, such like Glutamic Acid Decarboxylase (antiGAD). Studies on the presence and clinical implications of antiGAD positivity in the general non-diabetic population are however few, especially in adults. We aimed to investigate these aspects prospectively in a large sample of non-diabetic adults from an all-population based cohort.

**Materials and methods:** The study was based on the second and third health surveys in Nord-Trøndelag county (HUNT2; 1996-98 and HUNT3; 2006-08) located in Norway. Altogether 37,059 adults > 20 years of age took part in both surveys, i.e. 40% of all adults in the county. Equal numbers of men and women (about 250 each) who were non-diabetic both at HUNT2 and 3, were randomly selected from different age groups (age 20-29, 30-34 etc. to 65+), to a total of 4496 individuals. AntiGAD was measured in serum from HUNT2 and cases positive for antiGAD were followed up 10-12 years later (HUNT3) by additional measurements of antiGAD. We also measured antiGAD in HUNT2 in 55 cases who had adult-onset autoimmune diabetes at HUNT3 but did not have diabetes in HUNT2. AntiGAD positivity was defined from cut-offs (>0.05ai) based on participation in the Diabetes Autoantibody Standardization Program, indicating 82% sensitivity and 99% specificity.

**Results:** In persistently non-diabetic individuals the prevalence of antiGAD positivity in HUNT2 was 1.69% (n=76). Prevalence was highest in the 30-34 (3.2 %, n=16) and 35-39 (2.4%, n=12) age groups. AntiGAD positivity was not associated with sex (n=40 men and n=36 women), first degree family history of diabetes (21% among negative vs. 21% among positive), smoking (25% vs. 23%), non-fasting glucose ( $5.2 \pm 0.92$  vs.  $5.2 \pm 0.78$  mmol/l, mean and SD) or BMI ( $26 \pm 4$  vs.  $26 \pm 3$  kg/m<sup>2</sup>). There was association with the presence of Thyroid Peroxide antibody positivity (p<0.025). AntiGAD positivity persisted at follow-up (HUNT3) in 35 out of 76 individuals (46%). Persistently non-diabetic subjects who were antiGAD positive at HUNT2 were compared with cases who were antiGAD positive and non-diabetic at HUNT2 but had diabetes at HUNT3 (n=34). The latter cases had higher frequency of first degree family history of diabetes (53% vs. 28%, p=0.01), had higher antiGAD titre both in HUNT2 (1.42 [0.07-1.98] vs. 0.08 [0.06-3.58], median [min-max], p<0.001) and HUNT3 (1.42 [0.07-1.98] vs. 0.08 [0.06-3.58], p<0.001), and higher non-fasting glucose both in HUNT2 ( $7.1 \pm 4.3$  vs.  $5.2 \pm 0.78$ , p=0.001) and HUNT3 ( $10.6 \pm 5.2$  vs.  $5.5 \pm 0.96$ , p<0.001) compared to those who did not develop diabetes.

**Conclusion:** AntiGAD positivity in long-term (10-12 years) non-diabetic individuals is partly consistent, is not associated with parameters related to diabetes, but associated with thyroid autoimmunity. AntiGAD positivity with later occurrence of diabetes is on the other hand associated with risk factors related to autoimmune diabetes.

*Supported by: The liaison committee of the Central Norway Regional Health Authority*

86

### Thyroid function and autoimmunity in early pregnancy and risk of gestational diabetes mellitus: the mother-child RHEA cohort in Crete, Greece

L. Chatzi<sup>1</sup>, P. Karakosta<sup>1,2</sup>, D. Alegakis<sup>3</sup>, V. Georgiou<sup>1</sup>, E. Fthenou<sup>1</sup>, A. Pappas<sup>4</sup>, D. Boumpas<sup>3</sup>, E. Castanas<sup>2</sup>, M. Kogevas<sup>5</sup>;

<sup>1</sup>Faculty of Medicine, Department of Social Medicine, Heraklion, <sup>2</sup>Faculty of Medicine, Department of Experimental Endocrinology, Heraklion,

<sup>3</sup>Department of Rheumatology, Faculty of Medicine, Heraklion, <sup>4</sup>Diabetic Clinic, Venizelio Hospital, Heraklion, <sup>5</sup>National School of Public Health, Athens, Greece.

**Background and aims:** Maternal thyroid dysfunction, especially in early pregnancy, may lead to adverse obstetric and birth outcomes. The association between thyroid aberrations in early pregnancy and the development of gestational diabetes mellitus (GDM) remains little explored. The purpose of this study was to determine the extent to which, thyroid dysfunction and autoimmunity in early pregnancy is a risk factor for GDM.

**Materials and methods:** The study used data from the prospective mother-child cohort "Rhea" study in Crete, Greece, 2007-2009. The analysis included 1170 women with singleton pregnancies, and complete data on pregnancy outcomes. Maternal serum samples were collected at the time of the first major ultrasound (Mean: 12 weeks, SD: 1.5). Thyroid function was assessed by quantitative analysis of serum TSH (thyroid stimulating hormone), free thyroxine (free T4), free triiodothyronine (free T3), and thyroid peroxidase, and thyroglobulin antibodies (TPO-Ab and TG-Ab) [IMMULITE 2000 immunoassay system (Siemens Healthcare Diagnostics)]. Population-trimester-specific reference intervals were used to categorize women with low, normal, and high thyroid hormones during pregnancy. TPO-Ab and TG-Ab were considered elevated if levels were  $\geq 35$  IU/mL and  $>40$  IU/mL, respectively. Pregnant women were screened for gestational diabetes mellitus (GDM) between 24 and 28 weeks of gestation, and GDM was defined by the criteria proposed by Carpenter and Coustan. Multivariable log-poisson regression models were used to estimate the effect of thyroid function and autoimmunity in early pregnancy on the risk of GDM after adjusting for confounders.

**Results:** High TSH values were present in 79 (6.7%) women, while 29 (2.5%) women had low TSH. A total of 153 (13%) and 83 (7%) women had elevated levels of TPO-Ab and TG-Ab, respectively. Women with high TSH levels had a two fold higher risk of GDM (Relative Risk (RR) = 2.0, 95% CI = 1.0 - 4.0) after adjustment for maternal age, education, BMI before pregnancy, parity, family history of thyroid disease, and smoking during pregnancy. Stronger associations were estimated for the combination of high TSH levels and thyroid autoimmunity in early pregnancy and the risk of GDM (RR: 3.8, 95% CI = 1.7 - 8.5).

**Conclusion:** These findings suggest that women with high TSH levels and thyroid autoimmunity in early pregnancy had higher risk for GDM.

*Supported by: NewGeneris, 6th Framework Programme; Chicos, 7th Framework Programme*

87

### Metabolic signals of seroconversion to islet autoantibodies in children at risk for type 1 diabetes

G. Kastenmüller<sup>1</sup>, J. Krumsiek<sup>1</sup>, M. Pflüger<sup>2,3</sup>, A. Ziegler<sup>2,3</sup>, M. Orešić<sup>4</sup>;

<sup>1</sup>Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany, <sup>2</sup>Institute of Diabetes Research, Helmholtz Zentrum München, Neuherberg, Germany, <sup>3</sup>Forschergruppe Diabetes, Klinikum rechts der Isar, Technische Universität München, Germany, <sup>4</sup>VTT Technical Research Centre of Finland, Espoo, Finland.

**Background and aims:** Current high-throughput metabolomics techniques allow the assessment and quantification of hundreds of metabolites in human blood serum. Recently, the analysis of 29 amino acids and 12 lipid clusters revealed (age-dependent) metabolic differences between autoantibody-positive and -negative children at high risk for type 1 diabetes. The clusters had been determined based on 511 lipid-related traits whereof 290 so-called unknown metabolites are not assigned to a specific lipid molecule. The individual molecules have not been considered so far. In order to identify the molecules that most specifically indicate seroconversion and that may, thus, play an important role in autoimmunity, we revisited the data analyzing all 540 metabolic traits.

**Materials and methods:** For this study, we selected children, with a type 1 diabetic parent, who developed persistent islet autoantibodies (n=35), or

remained autoantibody-negative ( $n=35$ ), and were matched for age, date of birth, and HLA genotypes. UPLC-MS was used to quantify amino acids and lipids in the autoantibody-positive and the matched -negative serum samples. We applied Lasso logistic regression for discriminating between the autoantibody-positive and -negative group based on their metabolic profile. Moreover, we calculated a Gaussian Graphical Model (GGM) to embed the distinctive metabolites into their biochemical context. All individual metabolites and metabolite ratios that were significantly associated with the case/control phenotype based on a  $t$ -test were further characterized by testing their association to the genotypes of the subjects regarding single nucleotide polymorphisms (SNPs) at 12 type 1 diabetes risk loci.

**Results:** Interestingly, autoantibody-positive and -negative individuals can be perfectly distinguished by Lasso logistic regression based on the quantities of only five metabolites. This includes the triglyceride TG (44:1) and four unknown metabolites. The metabolite most relevant for the distinction of the two groups, namely an unknown metabolite with 310.2358  $m/z$ , showed largely different serum levels in autoantibody-positive ( $1.64 \pm 0.35 \mu\text{mol/l}$ ) and -negative ( $0.93 \pm 0.26 \mu\text{mol/l}$ ) individuals, making it a good candidate biomarker for seroconversion. For two of the four unknown metabolites, the GGM suggested a biochemical link to proline and tryptophan. Based on the important role of tryptophan catabolism in immunosuppression, we speculate that these unknowns might be involved in the regulation of tryptophan degradation. We further investigated the effect of genotypes at 12 diabetes risk loci onto the metabolite quantities in serum. Thereby, we revealed a possible link between various sphingomyelins and genetic variation in genes related to T cell receptor signaling (CTLA4, PTPN22). This includes a previously unknown metabolite that we identified and experimentally verified as the sphingomyelin SM (d18:2/22:0).

**Conclusion:** The detailed analysis of a metabolomics data set including unknown metabolites and the combination with genotyping data revealed novel molecular markers for the seroconversion to islet autoantibodies preceding type 1 diabetes.

*Supported by:* EP7-Health-2007, DIAPREPP N202013, FKZ 01GI0805-07, JDRF 1-2006-665

## 88

### The type 1 diabetes-associated common polymorphism in IFIH1 may affect the likelihood of enterovirus viraemia

**O. Cinek<sup>1</sup>**, G. Tapia<sup>2</sup>, E. Witso<sup>2</sup>, L. Kramna<sup>1</sup>, K. Holkova<sup>1</sup>, T. Rasmussen<sup>2</sup>, L.C. Stene<sup>3</sup>, K.S. Ronningen<sup>3</sup>

<sup>1</sup>Department of Pediatrics, 2nd Faculty of Medicine, Charles University in Prague, Czech Republic, <sup>2</sup>Norwegian Institute of Public Health, Oslo, Norway, <sup>3</sup>Oslo University Hospital, Norway.

**Background and aims:** The rs1990760, a common single nucleotide polymorphism in the IFIH1 gene (encoding for interferon induced with helicase C domain 1) has been confirmed to modify the risk of type 1 diabetes (T1D). Its putative underlying mechanism involves sensing the double-stranded RNA during virus replication - this provides a potential link to enterovirus. We therefore investigated whether the rs1990760 polymorphism influences the rate of viraemia.

**Materials and methods:** Enterovirus RNA was tested in 1001 blood samples, each from an infant or toddler (taken at median age 12.3 months, IQR 9.2 - 13.5, 512 males / 489 females) recruited from the general Norwegian population in the MIDIA study (646 with the highest-risk HLA genotype for T1D, 355 with other HLA genotypes). The rs1990760 was in Hardy-Weinberg equilibrium, with frequencies of 15.7%, 48.5% and 35.8% for Ala/Ala, Ala/Thr and Thr/Thr genotypes, respectively. The viraemia was tested using qualitative nested real-time reverse transcriptase PCR on RNA extracted from frozen cell packs after removal of plasma. Stool samples collected along with the blood sample (in a time window spanning 30 days before to 15 days after the date of blood sample) were available in 417 of the individuals, having been previously analyzed for enterovirus using a quantitative real-time single-tube reverse-transcriptase PCR.

**Results:** Enterovirus RNA was present in 11.5% blood samples; the positivity rate depended on age (dropping steeply after the first year of age), on the calendar year, and marginally on the season. We then constructed a regression model having enterovirus viraemia as the outcome, with the IFIH1 genotypes and known modifiers (calendar year, age of the child) as the predictors. It suggested an influence of the IFIH1 946Ala/Thr heterozygosity (OR=2.2, 95%CI 1.1 - 4.2,  $p=0.023$ , relative to the Ala/Ala genotype), while the Thr/Thr homozygosity did not affect the risk of viraemia ( $p=0.42$ ). This effect remained unchanged in the model restricted to individuals who had the concomitant

stool samples tested for enterovirus (OR of the Ala/Thr 2.2,  $p=0.07$ ), and was apparently independent of the enterovirus positivity in the stool (enterovirus in the gut further increased the likelihood of viraemia, OR=2.2, 95%CI 1.3-3.9,  $p=0.007$ ).

**Conclusion:** We observed that the rs1990760 polymorphism, previously confirmed to be associated with the risk of T1D, may affect the likelihood of enterovirus viraemia; this effect is however not straightforward, as we detected a kind of 'molecular heterosis' (a larger effect of heterozygosity than of either homozygous genotype). Molecular heterosis has been repeatedly noticed in humans, so the association may be in line with the presumed function of IFIH1 in the T1D pathogenesis. Its role is further supported by the independence of the association from the enterovirus positivity in the concomitant stools.

*Supported by:* IGA MZ Czech Republic NT11465, NRCN Norway 135893/330, 155300/320, 156477/7

## 89

### Detection of enteroviral protein expression and cellular antiviral responses in the islets of type 1 diabetes patients: a comparative analysis of two cohorts

**S.J. Richardson<sup>1</sup>**, A.J. Bone<sup>2</sup>, A.K. Foulis<sup>3</sup>, N.G. Morgan<sup>1</sup>

<sup>1</sup>Endocrine Pharmacology, Peninsula College of Medicine and Dentistry, Plymouth, <sup>2</sup>School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, <sup>3</sup>Department of Pathology, Glasgow Royal Infirmary, UK.

**Background and aims:** Enteroviral infections have been implicated in the initiation of beta-cell destruction in human Type 1 diabetes (T1D) and the presence of both the enteroviral capsid protein, vp1 and the pathogen recognition receptor, protein kinase R (PKR) was demonstrated in serial islet sections from a UK cohort of recent-onset type 1 diabetic patients. A more-recently established cohort of longer duration type 1 diabetes cases (JDRF nPOD; USA) and non-diabetic controls were examined, to determine whether the islet endocrine cells also display evidence of enteroviral infection and a comparison made with the UK cohort.

**Materials and methods:** Formalin-fixed paraffin embedded pancreatic sections from nPOD cases (17 type 1 diabetes and 12 controls) were examined by immunohistochemistry for the presence of vp1 (Dako antibody; 5D8/1), insulin, PKR and class I MHC. Results were compared with those of a previously analysed UK cohort, consisting of 72 cases of recent-onset type 1 diabetes and 119 non-diabetic controls.

**Results:** Multiple intensely vp-1 positive islet cells were observed in many insulin-containing islets (ICI) in 8 of the 17 nPOD type 1 diabetes cases. Of the 9 remaining cases that did not stain positively for vp1, 7 were immunonegative for insulin (i.e. they were devoid of ICI). Hence, expression of vp1 was detected in the islet cells of 8 of the 10 nPOD cases (80%) with ICIs present. By contrast, only 4/12 (33%) non-diabetic nPOD controls showed evidence of vp1 positive islet cells and, even where these did occur, the absolute number of immunopositive cells was very low. Indeed, among the controls, we found only 1 case with a total of more than 5 vp1+ islet cells in the entire pancreas section. This compares with the type 1 diabetes cases where large numbers of vp1+ islets cells were observed both within individual islets and across multiple islets. Despite the concordance between the nPOD and UK cohorts, differences were noted when comparing pancreas samples recovered from organ donors and those obtained at autopsy. A marked increase was seen in the number of individual endocrine cells within any given islet that were immunopositive for vp1 among the organ donor samples ( $5.3 \pm 0.6\%$  vs  $1.8 \pm 0.3\%$ ;  $p<0.001$ ) and this was independent of the source of the material (whether from nPOD or the UK cohort). The results reveal that enteroviral infection occurs commonly in the  $\beta$ -cells in human type 1 diabetes in two entirely separate cohorts. Additional studies using immunofluorescence approaches revealed that expression of vp1 always correlated with increased PKR expression in the islets of both cohorts, and that vp1 and PKR were invariably present within the same individual beta-cells. This is consistent with the concept that enteroviral infection leads to the development of an anti-viral response in islet cells.

**Conclusion:** Enteroviral vp1 expression is observed at high frequency in the ICI of type 1 diabetes cases in both the nPOD collection and the (older) UK cohort. It appears that, regardless of disease duration the presence of viral proteins correlates with the presence of residual beta cells. In both cohorts, enteroviral vp1 expression correlated with increased expression of the pathogen-recognition receptor, PKR and with hyper-expression of class I MHC.

*Supported by:* DRWF Non-Clinical Research Fellowship, JDRF nPOD, PEVNET



## 90

**Enterovirus genome and infectivity in peripheral blood leukocytes of children at the clinical onset of type 1 diabetes**A. Toniolo<sup>1</sup>, M. Colombo<sup>1</sup>, G. Bianchi<sup>1</sup>, A. Salvatori<sup>1</sup>, G. Federico<sup>2</sup>, A. Baj<sup>1</sup>;<sup>1</sup>Clinical and Experimental Medicine, University of Insubria Medical School, Varese, <sup>2</sup>Reproductive Medicine, University of Pisa Medical School, Italy.

**Background and aims:** Enterovirus (EV) infections are regarded as prominent environmental factors in the early stages of type 1 diabetes (T1D). On the day of clinical diagnosis of T1D, EV genome and infectivity were searched for in total peripheral blood leukocytes (PBL) of pediatric patients.

**Materials and methods:** This observational study was approved by the local Ethics Committee. PBL were obtained from 114 children on the day of T1D diagnosis at two Pediatric Endocrinology Centers in Italy (median age 9.0 yrs; range 2–16 yrs). EV-susceptible cell lines (RD, HeLa, AV3, CaCo) were co-cultured with the patients' PBL. Primers covering the 5' UTR, VP4, and 3D genome regions of EV of the A, B, C, and D species ( $\geq 100$  types) were used in highly sensitive RT-PCR assays that were run both on plasma and tissue culture medium from cell cultures exposed to patients' PBL. Expression of viral capsid proteins was evaluated in infected cell cultures using antiviral mAbs directed to the capsid protein VP1. Routine methods were used to measure levels of blood glucose, HbA1c, C-peptide (time 0 and 6 min after glucagon stimulation), diabetes related auto-Abs (GAD65, IA2, ZnT8, IAA), and - one year after diagnosis - the insulin requirement (IU/Kg/day). Immunoassays were used to quantify cytokines released by cultured cells. On the day of diagnosis, EV genome and infectivity were also searched for in consenting family members of 20 children.

**Results:** EV genome fragments and infectivity were detected in PBL of 90/114 (79%) children, versus 3/75 (4.0%) matched non-diabetic controls. Preliminary EV identification based on sequences of the 3Dpol genome region showed that EV of the B species were predominant (58% of positives). Viruses of the A, C, and D species were also detected. Tests on cell lines exposed to patients PBL confirmed the intracellular production of viral capsid antigen (immunofluorescence and WB). As compared to cell cultures exposed to controls' PBL, cell lines that had been exposed to patients' PBL released enhanced levels of the MCP1 chemokine. At the time of diagnosis, EV-positive patients had significantly higher levels of glucose and HbA1c as compared to EV-negative diabetic children. EV-positive children had also significantly reduced levels of glucagon-stimulated C-peptide. One year post-diagnosis, the insulin requirement was not different between the two groups. In a consenting cohort, EV genome and infectivity were found in blood of 18/20 (90%) diabetic children, 12/17 (70%) non-diabetic asymptomatic siblings, and 17/28 (61%) asymptomatic parents. Virus-positive members of each family shared the same EV species.

**Conclusion:** a) Detection of EV in blood represents a frequent biomarker of early stage T1D; b) EV of different species can be detected in newly-diagnosed patients (i.e., different EV species can associate with the early clinical stages of T1D); c) EV activity is expressed in human cell lines both as production of viral antigen and enhanced release of an inflammatory chemokine; d) EV genome and infectivity can be found both in newly diagnosed children and in their non-diabetic family members. This observation indicates that EV infections are spreading within the family at the early clinical stages of T1D. *Supported by:* CARIPO 2009-2577, VIDIS Group and Gianni Valcavi, Attorney

## OP 16 Mechanisms of insulin action

## 91

**Aerobic glycolysis is a major regulator of Akt activity**

S. Trefely, S. Tan, D.E. James;

Diabetes and Obesity, Garvan Institute of Medical Research, Sydney, Australia.

**Background and aims:** Tumour cells exhibit inordinately high levels of aerobic glycolysis, a phenomenon known as the Warburg effect. While the functional relevance of this effect is not known it is partly driven by upregulation of the enzyme PFKFB3 (6-Phospho-2-Fructo Kinase/Fructose-2,6-Bisphosphatase), which controls the synthesis of fructose-2,6-bisphosphate, an allosteric activator of glycolysis. Intriguingly, adipocytes exhibit the highest expression of PFKFB3 among most mammalian cell types. In view of the important role of the fat cell in nutrient sensing we wondered if PFKFB3 might play a role in this sensory mechanism. Consistent with previous studies we showed that fat cells apportioned the majority of their glucose uptake toward glycolysis. To test the role of PFKFB3 as a fuel sensor in adipocytes we examined the consequences of perturbing its activity and/or expression levels on glucose metabolism and insulin signalling.

**Materials and methods:** Insulin signalling was examined by immunoblot. Glucose uptake was measured using [<sup>3</sup>H]-2-deoxy-glucose. GLUT4 translocation was measured by cell surface labelling of HA-GLUT4 expressing cells. Lactate production was measured by extracellular flux analyser. The specific inhibitor of PFKFB3, 3PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one), was used to inhibit glycolysis. Overexpression of PFKFB3 and the bisphosphatase domain of PFKFB2 (BP-2) was used to increase or inhibit glycolysis, respectively. 3T3-L1 cells were electroporated with siRNA targeting the PFKFB3 sequence.

**Results:** Incubation of adipocytes with the PFKFB3 inhibitor 3PO led to a dose dependent decrease in insulin-stimulated glucose uptake and GLUT4 translocation. 100  $\mu$ M 3PO inhibited glucose uptake and GLUT4 translocation by 70% ( $p < 0.01$ ) and 50% ( $p < 0.01$ ) at sub-maximal dose (10  $\mu$ M). To further investigate the relationship between PFKFB3 activity and Akt signalling we examined the effect of over-expression or siRNA knock down of PFKFB3, or overexpression of the PFKFB3 antagonist BP-2, in HEK-293 cells. Knock down of PFKFB3 or BP-2 overexpression decreased insulin-stimulated Akt phosphorylation at Ser473 by 50% while PFKFB3 overexpression potentiated insulin-stimulated Akt phosphorylation increasing it 4 fold.

**Conclusion:** These studies reveal a novel mechanism for controlling Akt activity involving glucose flux via the glycolytic pathway. This pathway has potential implications for both cancer, where Akt activity may be driven by over stimulation of glycolysis, and diabetes where impaired glucose uptake may compromise Akt activity. The 'feedforward' mechanism by which insulin stimulation increases glycolysis is well described. Our study reveals a 'feedback' mechanism by which a defect in glycolysis decreases growth factor signalling through Akt.

## 92

**Upregulation of miRNA-143 expression by secretory products from epicardial adipose tissue from patients with type 2 diabetes abrogates insulin action in cardiomyocytes**M. Blumensatt<sup>1</sup>, S. Greulich<sup>1</sup>, B. Maxhera<sup>2</sup>, D. Herzfeld de Wiza<sup>1</sup>, P. Akhyari<sup>2</sup>, D.M. Ouwens<sup>1</sup>;<sup>1</sup>Institute of Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, <sup>2</sup>Department of Cardiovascular Surgery, Heinrich-Heine-University, Duesseldorf, Germany.

**Background and aims:** Cardiac dysfunction and myocardial insulin resistance are common in patients with type 2 diabetes. We have recently found that conditioned media (CM) generated from epicardial adipose tissue from patients with type 2 diabetes (CM-EAT-T2D) abrogate insulin action in primary rat cardiomyocytes. This was not observed in cardiomyocytes exposed to CM-EAT from patients without type 2 diabetes (CM-EAT-ND) and from subcutaneous (SAT) and pericardial adipose tissue (PAT) from the same patients. In this study, we examined whether the induction of insulin resistance can be ascribed to alterations in miRNA expression induced by CM-EAT-T2D.

**Materials and methods:** Biopsies from EAT, PAT and intrathoracic SAT were collected from males of Caucasian origin undergoing open heart surgery.

Participants were distributed into two groups, ND or T2D, on the basis of the diagnosis T2D in the patient's medical record. Conditioned media (CM) were generated from these biopsies from 7 different donors of each group. Primary adult rat cardiomyocytes were exposed to different CMs, and examined for alterations in the expression of 364 microRNAs by real-time PCR. The cardiac mouse cell line HL-1 was used for transfection with precursor-miRNAs to investigate the impact of miRNAs on insulin signalling. Effects on protein expression and phosphorylation were examined by Western blot analysis.

**Results:** Profiling miRNA expression in primary adult rat cardiomyocytes incubated with CM-EAT-T2D identified alterations in the expression of several miRNA species, including upregulation of let 7c (1.3 -fold), and the miRNA-143/145 cluster (1.4-fold), as compared to CM-EAT from patients without type 2 diabetes and CM generated from SAT and PAT from the same patients ( $P<0.05$ ). Validation of these miRNA species in HL-1 cardiomyocytes identified miRNA-143 as modulator of insulin action. Accordingly, transfection of HL-1 cardiomyocytes with a precursor of miR-143 (pre-miR-143) blunted the phosphorylation of Akt at Ser473 and Thr308 and its substrate proline-rich Akt substrate of 40-kDa (PRAS40) by insulin by 25% ( $P<0.05$ ). In addition, expression of pre-miR-143 reduced insulin-mediated GLUT4 translocation to the sarcolemma by 20% ( $P<0.05$ ), and insulin-mediated glucose uptake by 25% ( $P<0.05$ ). These effects were paralleled by a 20% reduction in protein levels of the miRNA-143 target and regulator of insulin action, the oxysterol binding protein-like 8 (ORP8) ( $P<0.05$ ).

**Conclusion:** Inhibition of insulin action in cardiomyocytes by secretory products from epicardial adipose tissue involves upregulation of miRNA-143. This miRNA inhibits the Akt-pathway regulating glucose uptake through downregulation of the novel regulator of insulin action, ORP8.

## 93

### Chronic carotid sinus nerve resection prevents the development of insulin resistance in rats fed with hypercaloric diets

M.J. Ribeiro, J.F. Sacramento, E.C. Monteiro, S.V. Conde;  
Farmacologia, CEDOC, Faculdade Ciências Médicas, Universidade Nova de Lisboa, Portugal.

**Background and aims:** The carotid bodies (CB) are peripheral chemoreceptors that respond to hypoxia by increasing the action potential frequency in their sensory nerve, the carotid sinus nerve (CSN), which increases minute ventilation and activates the sympathetic nervous system. Since increased sympathetic nervous system activity is a well-known pathophysiological mechanism for hypertension and insulin resistance (IR), herein we tested if blockade of CB activity through CSN resection prevents the development of IR in rats submitted to high fat (HF) and high sucrose (HSu) diets.

**Materials and methods:** Six groups of Wistar rats, aged 9–12 weeks were used. The control group was fed a sham diet (7.4% fat + 75% carbohydrate (4% sugar) + 17% protein); the HSu and HF models were obtained by the administration of 35% sucrose in drinking water during 28 days; or of a lipid rich diet (45% fat + 35% carbohydrate + 20% protein) during 21 days, respectively. Contribution of CB to the development of IR was assessed by submitting all the groups of animals to bilateral chronic CSN resection, 5 days prior to the beginning of the HF or HSu diet protocol. The control group was fed with regular chow and submitted to a sham surgical procedure. Surgical procedure was performed under the follow anaesthesia/recovery protocol: ketamine (30mg/kg) plus xilazine (4mg/kg) plus buprenorphine (10µg/kg). Experiments were performed at the end of the treatments under pentobarbital anaesthesia. Insulin sensitivity was measured through the insulin tolerance test (ITT). Blood pressure, weight, visceral and total fat, basal glycaemia, insulinemia, free fatty acids and corticosterone were also measured.

**Results:** Insulin sensitivity diminished in sham HF and HSu rats as the constant of the insulin tolerance test ( $K_{ITT}$ ) decreased significantly to  $2.82\pm0.32$  and  $2.23\pm0.29$  compared to the control value  $4.81\pm0.39$ . CSN resection in HF and HSu animals prevented the development of IR since  $K_{ITT}$  return to control values. Basal glycaemia was significantly increased in HSu but not in HF rats, and CSN resection restored the values to control levels. Insulinemia was significantly increased by 137.1% and 172.9% in HF and HSu rats, respectively and CSN resection completely abolished these increases. Non-esterified free fatty acids were increased in HSu animals and CSN denervation returns those values to control. Corticosterone levels were not modified by HF, HSu nor by bilateral CSN cut.

**Conclusion:** Our results suggest that CB is involved in the development of insulin resistance induced by hypercaloric diets.

Supported by: FCT-PTDC/SAU-ORG/111417/2009

## 94

### Resveratrol treatment restores peripheral insulin action in diabetic IRS2-deficient mice in a Sirt1-independent manner

Á. González-Rodríguez<sup>1,2</sup>, J.A. Mas-Gutiérrez<sup>3</sup>, V. Pardo<sup>1,2</sup>, E. Fernández-Millán<sup>1,4</sup>, A.I. Arroba<sup>1,2</sup>, B. Santamaría<sup>1,2</sup>, M.A. Mobasher<sup>1,2</sup>, C. Álvarez<sup>4,1</sup>, M. Ros<sup>3</sup>, Á.M. Valverde<sup>1,2</sup>;  
<sup>1</sup>CIBERDEM, <sup>2</sup>Instituto de Investigaciones Biomédicas “Alberto Sols” (CSIC/UAM), <sup>3</sup>Universidad Rey Juan Carlos, <sup>4</sup>Universidad Complutense, Madrid, Spain.

**Background and aims:** Mice with complete deletion of insulin receptor substrate (IRS) 2 develop hyperglycaemia, impaired hepatic insulin signalling and elevated gluconeogenesis. The elevated expression and activity of PTP1B in the liver of hyperglycaemic IRS2<sup>-/-</sup> mice block insulin receptor (IR)/IRS1-mediated insulin signalling by increasing the association of IR with this phosphatase. Accordingly, PTP1B inhibition by genetic (double mutant IRS2<sup>-/-</sup>-PTP1B<sup>-/-</sup>) and pharmacological (resveratrol treatment) strategies promotes insulin sensitivity in the liver of IRS2<sup>-/-</sup> mice through the restoration of IRS1-mediated Akt/Foxo1 phosphorylation and the inhibition of gluconeogenic enzymes. Moreover, resveratrol, a plant-derived polyphenolic compound, has been receiving increasing attention as a potent activator of the histone deacetylase Sirt1 with anti-oxidant and anti-neoplastic properties. In fact, recent studies have demonstrated that both resveratrol treatment and moderate increase of Sirt1 levels improved insulin sensitivity. On that basis, the purpose in this study was to investigate if resveratrol action on IRS2<sup>-/-</sup> mice is mediated by Sirt1 activation. For this goal, we have generated IRS2<sup>-/-</sup> mice with moderate over-expression of Sirt1.

**Materials and methods:** We characterized whole body glucose homeostasis, insulin sensitivity and insulin signalling in liver and muscle of hyperglycaemic IRS2<sup>-/-</sup> deficient mice, IRS2<sup>-/-</sup> mice treated with resveratrol (IRS2<sup>-/-</sup>-Resv) and IRS2<sup>-/-</sup> mice that moderate over-express Sirt1 (IRS2<sup>-/-</sup>-Sirt1). mRNA levels, protein expression and enzymatic activity of PTP1B in liver and muscle have been analyzed.

**Results:** Resveratrol treatment improved systemic insulin sensitivity in hyperglycaemic IRS2<sup>-/-</sup> mice but did not change glucose tolerance due to the inability to revert beta cell failure. On the other hand, moderate over-expression of Sirt1 in IRS2<sup>-/-</sup> mice did not recover neither peripheral insulin resistance nor glucose intolerance. In both liver and muscle of hyperglycaemic IRS2<sup>-/-</sup> mice levels of PTP1B were increased. Resveratrol treatment of IRS2<sup>-/-</sup> mice significantly decreased PTP1B mRNA and inhibited its activity in both tissues, thereby restoring IRS1-mediated PI3kinase/Akt signalling. Conversely, moderate over-expression of Sirt1 could not normalized PTP1B levels and, consequently, insulin signalling in liver and muscle remained impaired.

**Conclusion:** In conclusion, our results have established that elevated PTP1B expression in liver and muscle of hyperglycaemic IRS2<sup>-/-</sup> mice impaired insulin signalling. Accordingly, PTP1B inhibition by resveratrol promotes insulin sensitivity in IRS2<sup>-/-</sup> mice through the restoration of IRS1-mediated signalling in peripheral tissues. Moreover, we have demonstrated that the effects of resveratrol on insulin action in IRS2<sup>-/-</sup> mice are not mediated through Sirt1 activation.

Supported by: EFSD/Amylin grant; SAF2009-08114 (MICINN) and CIBERDEM (ISCIII), Spain

## 95

### Local effects of cytokine TNF-alpha on glucose, protein and lipid metabolism in the placebo controlled bilaterally perfused human leg

E. Bosnjak<sup>1</sup>, R.R. Nielsen<sup>2</sup>, M. Buhl<sup>3</sup>, M.H. Vendelbo<sup>1</sup>, E. Tønhesen<sup>4</sup>, N. Møller<sup>1</sup>;

<sup>1</sup>Medical Department MEA, Aarhus University Hospital, NBG, Aarhus,

<sup>2</sup>Department of Cardiology, Aarhus University Hospital, Skejby,

<sup>3</sup>Department of Pediatrics, Aarhus University Hospital, Skejby, <sup>4</sup>Department of Anesthesia and Intensive Care Medicine, Aarhus University Hospital, NBG, Aarhus, Denmark.

**Background and aims:** Cytokine tumor necrosis factor alpha (TNF-alpha) has widespread metabolic actions, including induction of insulin resistance and cachexia. Systemic TNF-alpha administration, however, generates complex hormonal and metabolic scenario. No studies employing regional, placebo controlled TNF-alpha infusion exist. Our study was designed to test the hypothesis that local leg perfusion with TNF-alpha directly induces insulin resistance, lipolysis and protein breakdown.

**Materials and methods:** We studied eight healthy volunteers with bilateral femoral vein and artery catheters during 3-h basal period and 3-h insulin stimulation (hyperinsulinemic euglycemic clamp). One femoral artery was perfused with saline and the other with TNF- $\alpha$  (Tasonermin, 6 ng/kg/h). Amino acid metabolism was quantified with 15N-Phenylalanine tracer, lipid metabolism with 3H-Palmitate tracer and arterio-venous differences of free fatty acids and glucose metabolism was quantified by arterio-venous differences.

**Results:** TNF- $\alpha$  perfusion significantly (measured by Two Way RM ANOVA) increased local leg glucose uptake during the clamp. Basal glucose arterio-venous difference was unaltered, but substantially increased during the clamp ( $p<0.001$ ), with  $0.925\pm0.49$  mmol/l in the TNF- $\alpha$  leg, and  $0.744\pm0.43$  mmol/l in the placebo leg. Net phenylalanine release was increased by TNF- $\alpha$  perfusion ( $p=0.023$ ). During the basal period there was an increased phenylalanine release ( $p=0.003$ ), with  $4466\pm1390$   $\mu\text{g}/\text{min}$  in the TNF- $\alpha$  leg, and  $3794\pm1122$   $\mu\text{g}/\text{min}$  in the placebo leg, and an increased phenylalanine uptake ( $p=0.023$ ) with  $2951\pm1134$   $\mu\text{g}/\text{min}$  in the TNF- $\alpha$  leg, and  $2464\pm708$   $\mu\text{g}/\text{min}$  in the placebo leg. Free fatty acids and Palmitate kinetics were not affected by TNF- $\alpha$ .

**Conclusion:** TNF- $\alpha$  directly increased muscle protein breakdown and local muscle insulin sensitivity in terms of increased muscle glucose uptake. The finding of increased muscle loss may contribute to general protein loss during severe illness. The finding of increased insulin sensitivity is unexpected and highlights the necessity of differentiation between direct local cytokine effects as in this study and those secondary to release of stress cascades including hormones such as epinephrine, glucagon and cortisol.

Clinical Trial Registration Number: NCT01452958

Supported by: Aarhus University, The Lundbeck Foundation

## 96

### Thrifty metabolic responses to 36 hours of fasting in young men with low compared to normal birth weight

S.W. Jørgensen<sup>1,2</sup>, L.J. Bluck<sup>3</sup>, C. Brøns<sup>1</sup>, K. Færch<sup>3</sup>, A. Thankamony<sup>3</sup>, D. Dunger<sup>4</sup>, A. Vaag<sup>1</sup>

<sup>1</sup>Diabetes and Metabolism, Rigshospitalet, Copenhagen, <sup>2</sup>Steno Diabetes Center A/S, Gentofte, Denmark, <sup>3</sup>MRC Human Nutrition Research, Cambridge, UK, <sup>4</sup>Department of Paediatrics, University of Cambridge, UK.

**Background and aims:** The thrifty metabolic phenotype associated with low birth weight (LBW) imposes an increased risk of type 2 diabetes in an affluent society, but may conversely confer improved survival during sparse living conditions. We hypothesized that otherwise healthy individuals born with LBW may display an improved cardiometabolic risk profile when exposed to short time fasting compared with normal birth weight (NBW) controls.

**Materials and methods:** The participants were recruited from the Danish National Birth Register. LBW was defined as a birth weight  $\leq$  10th percentile and NBW between the 50th and the 75th percentile of the birth cohort. Eighteen NBW and 21 LBW young healthy male individuals, matched for age and BMI, were recruited and subjected to a 36-hour fast, followed by a frequently sampled intravenously glucose tolerance test (FS-IVGTT) to allow for minimal modelling. Glucose, insulin and lipids were collected frequently during the study. A [6,6-D<sub>2</sub>]-2H<sub>2</sub> glucose tracer was used to assess the rate of appearance of glucose (Ra) after 36 hours of fasting. [6,6-D<sub>2</sub>]-2H<sub>2</sub> glucose and insulin were included in the FS-IVGTT to assess glucose mediated glucose uptake (Sg) and insulin sensitivity (Si). Indirect calorimetry was obtained after 12, 31 and 34 hours of fasting.

**Results:** Serum insulin levels decreased significantly more in LBW compared with NBW subjects during the 36 hours fasting ( $p<0.001$ ). In addition, energy expenditure decreased in LBW subjects ( $P=0.11$ ) whereas it increased in NBW subjects during the fasting period ( $P=0.020$ ). LBW subjects displayed a lower fat oxidation rate than NBW subjects after fasting ( $P=0.015$ ). Rate of appearance of glucose (Ra) ( $P=0.046$ ) and glucose mediated glucose uptake (Sg) ( $P=0.054$ ) was lower in LBW compared with NBW subjects after fasting, whereas insulin sensitivity (Si) was not significantly different between groups. Plasma triglyceride levels decreased significantly more among LBW compared with NBW subjects after 36 hours of fasting ( $P<0.02$ ). After the IVGTT, excretion of glucose in the urine was disproportionately reduced in the LBW subjects ( $P=0.027$ ).

**Conclusion:** Young healthy men born with LBW displays a differential and more beneficial response of their cardiometabolic risk profile during 36 hours of fasting compared with those born with NBW. These results are consistent with the “thrifty phenotype hypothesis”, and the notion that LBW subjects are better to conserve energy during energy restricted conditions.

Supported by: EFSD Clinical Research Grant

## OP 17 Mitochondria: signalling power plants of the beta cell

### 97

#### Deficiency of TFB1M leads to beta cell dysfunction and development of diabetes

V.V. Sharoyko, M. Ländin, I. Mollet, P. Spégel, L. Eliasson, N. Wierup, H. Mulder;

Clinical Sciences, Lund University, Malmö, Sweden.

**Background and aims:** Recently, we have used information from Genome-wide Association Studies of type 2 diabetes (T2D) and its associated traits, and identified a novel potential culprit in the pathogenesis of the disease: Transcription/translational Factor B1 Mitochondrial (TFB1M). This protein controls mitochondrial protein translation and therefore the expression of mitochondrially encoded genes. We observed that carriers of a common variant of *Tfb1m* are at increased risk of developing T2D, due to impaired insulin secretion and elevated glucose levels during an oral glucose tolerance test. Perturbed mitochondrial metabolism and impaired insulin secretion were found in *Tfb1m*<sup>-/-</sup> mice. To resolve the regulatory role of TFB1M in  $\beta$ -cells and its pathogenetic role in T2D islets, we performed *in vivo* and *in vitro* studies in a murine model of  $\beta$ -cell-specific *Tfb1m* deficiency ( $\beta$ -*Tfb1m*<sup>-/-</sup>).

**Materials and methods:** To create  $\beta$ -cell *Tfb1m*<sup>-/-</sup> mice, RIP-cre transgenic mice were cross-bred with floxed *Tfb1m*<sup>+/-</sup> mice.  $\beta$ -cell-specific knock out of TFB1M was assessed by qRT-PCR, primer extension analysis of 12S rRNA dimethylation. The OCR (oxygen consumption rate) was measured using the extracellular flux analyser XF24. ATP measured with a Luciferase-based luminescent assay. Ultrastructure analysis of insulin granules and mitochondria in  $\beta$ -cells within isolated islets was performed by electron microscopy. Monoclonal antibody MOMA-2 was used for immunocytochemical detection of macrophages in islets.

**Results:** The islets from *-Tfb1m*<sup>-/-</sup> mice were studied before onset of diabetes. Insulin secretion from islets in response to a rise from 2.8 to 16.7 mM glucose was significantly reduced compared to that in controls. *-Tfb1m*<sup>-/-</sup> islets showed an OCR rate at the basal glucose level and failed to increase their OCR in response to elevation of the glucose concentration. We also found a pronounced decrease in the rate of mitochondrial ATP production from *-Tfb1m*<sup>-/-</sup> islets compared to their controls. The volume density of insulin granules, which is a measure of the total number of granules, was reduced from  $10.7\pm0.7$  granules/ $\mu\text{m}^3$  in the control mice to  $4.7\pm1.1$  granules/ $\mu\text{m}^3$  in the  $\beta$ -*Tfb1m*<sup>-/-</sup> mice. The surface density of insulin granules, which is a measure of docked granules, was reduced from  $0.64\pm0.07$  granules/ $\mu\text{m}^3$  in the control mice to  $0.34\pm0.04$  granules/ $\mu\text{m}^3$  in the  $\beta$ -*Tfb1m*<sup>-/-</sup> mice. However, the fraction docked granules per total number of granules increased, why the reduction in docking is a consequence of the reduced number of granules. The granule diameter was  $317\pm7$  nm in control  $\beta$ -cells and  $385\pm9$  nm in the  $\beta$ -*Tfb1m*<sup>-/-</sup>  $\beta$ -cells. In the  $\beta$ -*Tfb1m*<sup>-/-</sup>  $\beta$ -cells area covered by mitochondria increased from  $7\pm2\%$  in the controls to  $19\pm9\%$  in the  $\beta$ -*Tfb1m*<sup>-/-</sup>. After 4 months of age, we found that virtually all  $\beta$ -*Tfb1m*<sup>-/-</sup> mice had developed diabetes and  $71\pm22\%$  of islets in  $\beta$ -*Tfb1m*<sup>-/-</sup> mice and  $32\pm8\%$  of islets in control mice were positive for macrophages.

**Conclusion:** Taken together our results obtained with the  $\beta$ -*Tfb1m*<sup>-/-</sup> mice suggest that *Tfb1m* deficiency plays a major role in  $\beta$ -cell function conferring to this protein, identified as a genetically associated T2D risk factor, a specific biological role in the pathogenesis of the disease. We suggest that the final effect on insulin secretion due to *Tfb1* deficiency may underline how perturbation in the mitochondrial proteome homeostasis is a condition which finally leads to the development of T2D. Further work will help in the validation of potentially new therapeutic targets.

Supported by: Swedish Research Council and EFSD/Lilly grant



## 98

**Fis1, a key regulator of the mitochondrial network, controls glucose responsiveness in beta cells**

J. Schultz<sup>1</sup>, A. Hempel<sup>1</sup>, R. Waterstradt<sup>1</sup>, A. Rieckmann<sup>1</sup>, V. Sharoyko<sup>2</sup>, H. Mulder<sup>2</sup>, M. Tiedge<sup>1</sup>, S. Baltrusch<sup>1</sup>;

<sup>1</sup>Institut of Medical Biochemistry and Molecular Biology, Rostock, Germany,

<sup>2</sup>Department of Clinical Sciences, Unit of Molecular Metabolism, Lund University Diabetes Centre, Malmö, Sweden.

**Background and aims:** Mitochondria exist in dynamic networks, and cycle continuously through fusion-fission events. Mitofusin 1 and 2 (Mfn1 and Mfn2) and the optic atrophy 1 protein (Opa1) are essential for mitochondrial fusion, whereas the fission protein 1 (Fis1) and the dynamin related protein 1 (Drp1) control fission. Because Fis1 determines the frequency of the fission process, this protein has been suggested to be a key regulator of mitochondrial dynamics. Moreover, impaired mitochondrial morphology in beta cells has been proposed to contribute to the pathogenesis of type 2 diabetes mellitus. Therefore, the aim of this study was to investigate mitochondrial dynamics in glucose-responsive and glucose-unresponsive INS1-derived beta cells with an emphasis on Fis1.

**Materials and methods:** Experiments were performed in INS1 832/13 (glucose-responsive) and INS1 832/2 (glucose-unresponsive) beta cells. Fis1 knockdown was evoked by the GIPZ lentiviral shRNAir system and Fis1 overexpression by transduction with the pLUX vector system. Lentiviral particles harbouring scrambled sequences served as control. Gene and protein expression were analyzed by quantitative real-time PCR, western blot and immunofluorescence analyses, respectively. ATP content was measured using the ATPlite assay and glucose-stimulated insulin secretion using ELISA. Mitochondrial morphology was determined after MitoTracker Green staining.

**Results:** In contrast to the efficient increase in the ATP concentration and insulin secretion in glucose-responsive cells, this response to a glucose stimulus was absent in glucose-unresponsive cells. Interestingly, in the glucose-unresponsive cells gene expression of Fis1, Drp1, Opa1, Mfn1 and Mfn2 was significantly reduced in comparison to glucose-responsive cells. Particularly the Fis1 protein level was significantly reduced in glucose-unresponsive cells. Consistent with the low Fis1 expression, we observed elongation and clustering of mitochondria in unresponsive cells. In contrast, glucose-responsive cells showed a homogenous mitochondrial network. To prove the hypothesis that Fis1 expression and the resulting mitochondrial structure significantly contribute to glucose responsiveness, Fis1 was knocked down in glucose-responsive cells. Indeed, we observed an elongation and clustering of mitochondria and a significant reduction of glucose-stimulated insulin secretion. We also demonstrated that overexpression of Fis1 in glucose-unresponsive cells improves the mitochondrial morphology and restores glucose-stimulated insulin secretion.

**Conclusion:** Our data suggest that the structure of the mitochondrial network in beta cells has an important impact on glucose-stimulated insulin secretion. We propose that Fis1 is a crucial factor in this process. Thus, the challenge of future studies will be the identification of regulators of Fis1 expression enabling selective counteraction of Fis1 down-regulation and loss of glucose responsiveness in beta cells.

*Supported by: Deutsche Diabetes Stiftung*

## 99

**Activation of the 5-HT2b receptor in INS-1 cells couples to mitochondrial respiration and potentiates glucose stimulated insulin secretion**

H. Bennet, A. Balhuizen, V. Sharoyko, C.L. Nagorny, M. Fex;

The unit of diabetes and celiac disease, Clinical science, Malmö, Sweden.

**Background and aims:** 5-Hydroxytryptamine (5-HT) activates a large family of receptors. Several 5-HT receptors (5-HT1a, 5-HT2b and 5-HT1d) have previously been shown to be expressed in rodent islets of Langerhans. 5-HT has been shown to be present and co-secreted with insulin from rodent beta-cells. As both receptors and amines are present within the islets, 5-HT may potentially regulate hormone secretion from islets of Langerhans. A selective 5-HT2b agonist (alpha-Methyl serotonin maleate salt (alpha-MET)) potentiates glucose stimulated insulin secretion, but was unable to enhance the secretory response at low concentrations of glucose. Therefore, we hypothesize that 5-HT2b receptor signalling may affect glucose oxidation and consequently mitochondrial respiration.

**Materials and methods:** We used quantitative (Q) PCR to detect expression of the receptor in a beta-cell line (INS 832/13). One hour static incubations were performed in INS (832/13) cells using either low (2.8 mM) or high (16.7 mM) glucose with the addition of the selective 5-HT2b agonist alpha-MET (5uM). Knock down of the receptor in INS (832/13) cells was performed using siRNA and insulin secretion was measured using insulin ELISA. Oxygen consumption rate (OCR) was measured using the Seahorse extracellular flux analyser XF24 in a 24-well format by sensing changes in oxygen content in a 7 µl volume above the plated cells with a fluorescence biosensor.

**Results:** We found 5-HT2b to be expressed in INS (832/13) cells at the mRNA level. Moreover, stimulating the receptor using a specific agonist alpha-MET, at high glucose (16.7 mM) for 1 hour static incubations, potentiated glucose stimulated insulin secretion (P-value=0.002). In addition, performing knock-down experiments using siRNA resulted in a 70% decrease in expression at the mRNA level, and a 30% reduction in insulin glucose stimulated insulin secretion (P-Value=0.006). Preliminary data, performed in INS (832/13) cells, show that the overall OCR was increased with the addition of a high glucose. After adding FCCP, a wellknown uncoupler of the respiratory chain, alpha-MET provoked an increase in OCR, significantly higher than glucose alone, thereby indicating that alpha-MET can enhance overall oxidative capacity of the cells.

**Conclusion:** In this study we show that alpha-MET, via activation of the 5-HT2b receptor potentiates glucose stimulated insulin secretion. Subsequent knock down of the receptor diminishes glucose stimulated insulin secretion. Moreover, we show that 5-HT2b signalling enhance mitochondrial respiration. Therefore, our results strongly suggest that the 5-HT2b receptor signalling couples to mitochondrial respiration.

*Supported by: The NOVO NORDISK foundation*

## 100

**Effects of catalase overexpression on insulin secretion, mitochondrial membrane potential and glucose-induced toxicity in insulin-secreting INS-1E cells**

S. Lortz, E. Gurgul-Convey, S. Lenzen;

Institute of Clinical Biochemistry, Hannover Medical School, Germany.

**Background and aims:** The expression of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) inactivating enzymes such as catalase in β-cells is extraordinary low and can explain their extreme susceptibility against reactive oxygen species (ROS) and proinflammatory cytokines. Whether this low expression pattern might be related to a signalling function of H<sub>2</sub>O<sub>2</sub> within the beta cell is unknown. A high expression level of H<sub>2</sub>O<sub>2</sub> inactivating enzymes could potentially be incompatible with glucose-stimulated insulin secretion (GSIS). In order to analyse the effect of catalase overexpression on mitochondrial function in insulin-secreting INS-1E cells effects on physiological insulin secretion were studied.

**Materials and methods:** The H<sub>2</sub>O<sub>2</sub>-inactivating enzyme catalase was overexpressed mitochondrially (MitoCat) or cytosolically (CytoCat) in insulin-secreting INS-1E cells. Successful overexpression was verified by measurement of catalase enzyme activity. The protective effect of catalase overexpression was quantified after cytokine treatment (IL-1β alone or a mixture of IL1β, TNF-α and IFN-γ) and hypoxic incubation. UCP2 expression was quantified by qRT-PCR, mitochondrial membrane potential was analysed with the fluorescence dye rhodamine 123, GSIS was measured after static glucose incubation (3, 10 and 30 mM) by RIA and the generation of ROS was detected by dichlorodihydrofluorescein diacetate (DCFDA-H<sub>2</sub>).

**Results:** The overexpression of catalase in both compartments resulted in a significant 14 - 17fold higher enzyme activity compared to controls. MitoCat overexpression protected INS-1E cells against cytokine- and hypoxia-mediated toxicity. Furthermore overexpression of MitoCat increased UCP2 expression significantly by 50 % while in CytoCat expressing INS-1E cells the UCP2 expression was unchanged. The mitochondrial membrane potential at 3 and 10 mM glucose was not affected by catalase overexpression, but at 30 mM MitoCat cells showed a significant reduction. Insulin secretion at 10 and 30 mM glucose was significantly higher compared with 0 mM and GSIS was not altered after catalase overexpression. Increasing glucose concentrations lead to a reduction of the ROS generation in control cells by 30 (10 mM) and 45 % (30 mM). At basal (3 mM) and elevated glucose concentrations (10 und 30 mM) MitoCat cells showed a 30 - 40% reduced ROS production.

**Conclusion:** The results suggest that the mitochondrial overexpression of catalase protects against proinflammatory cytokines and hypoxia. Although the expression of UCP2 was elevated in parallel, mitochondrial membrane potential and glucose-stimulated insulin secretion at physiological glu-

cose concentrations were not blunted, indicating that  $\text{H}_2\text{O}_2$  had no second messenger function for the insulin secretion signalling pathway and that a moderate uncoupling of the respiratory chain can be tolerated without loss of function. Elevated glucose concentrations did not result in a higher ROS generation rate, but instead in a reduction. The further reduction of the ROS generation by MitoCat and the missing effect of CytoCat overexpression indicate that the glucose-dependent ROS production is mitochondrially located. Therefore the mitochondrial catalase overexpression could protect pancreatic  $\beta$ -cells against cytokine-mediated  $\beta$ -cell destruction during autoimmune diabetes onset and after islet transplantation without interfering with physiological insulin secretion.

## 101

### The mitochondrial $\text{Ca}^{2+}$ uniporter MCU is essential for glucose and sulfonylurea sensing by mouse pancreatic beta cells

A.I. Tarasov<sup>1</sup>, F. Semplici<sup>1</sup>, M.A. Ravier<sup>2</sup>, E.A. Bellomo<sup>1</sup>, T.J. Pullen<sup>1</sup>, P. Gilon<sup>3</sup>, I. Sekler<sup>4</sup>, R. Rizzuto<sup>5</sup>, G.A. Rutter<sup>1</sup>;

<sup>1</sup>Department of Medicine, Imperial College London, UK, <sup>2</sup>Université Montpellier, France, <sup>3</sup>Université Catholique de Louvain, Brussels, Belgium,

<sup>4</sup>Department of Medicine, Ben Gurion University, Beer-Sheva, Israel,

<sup>5</sup>University of Padua, Italy.

**Background and aims:** Glucose induces insulin release from pancreatic  $\beta$ -cells by stimulating ATP synthesis, membrane depolarisation and  $\text{Ca}^{2+}$  influx. As well as activating ATP-consuming processes, cytosolic  $\text{Ca}^{2+}$  increases may also be transmitted into mitochondria to potentiate ATP synthesis. Whilst a candidate mitochondrial  $\text{Ca}^{2+}$  uniporter, MCU, encoded by *ccdc109a*, has recently been identified, its role in the pancreatic  $\beta$ -cell has yet to be explored. Here, we have silenced MCU in primary mouse  $\beta$ -cells and examined the role of this protein in mitochondrial  $\text{Ca}^{2+}$  transport, ATP synthesis and insulin secretion.

**Materials and methods:** We have combined patch-clamp electrophysiology with real-time imaging of compartmentalised changes in  $\text{Ca}^{2+}$  and ATP/ADP ratio in single primary mouse  $\beta$ -cells, using adenovirally-expressed recombinant targeted (Pericam, Perceval) or entrapped intracellular (Fura Red) probes. Insulin exocytosis was monitored in single cells with the extracellular membrane-bound  $\text{Zn}^{2+}$ -sensitive dye, ZIMIR. Expression of MCU was reduced through the use of lentivirally-delivered short-hairpin RNAs.

**Results:** Silencing of MCU resulted in a  $58 \pm 15\%$  ( $p < 0.05$ ) reduction of the depolarisation-induced mitochondrial  $\text{Ca}^{2+}$  increases but had no effect on cytosolic  $\text{Ca}^{2+}$  changes. In response to 16.7 mmol/l glucose, MCU-depleted cells displayed a normal first phase of cytosolic ATP/ADP ratio increase. However, the second (sustained) phase of ATP/ADP elevation showed  $42 \pm 8\%$  ( $p < 0.05$ ) inhibition in these cells. Finally, sulfonylurea-induced insulin secretion was markedly ( $67 \pm 17\%$ ,  $p < 0.02$ ) inhibited in the absence of MCU.

**Conclusion:** MCU is critical for mitochondrial  $\text{Ca}^{2+}$  uptake in pancreatic  $\beta$ -cells and thereby for the ability of these cells to respond to glucose with an appropriate activation of mitochondrial oxidative metabolism and ATP synthesis. Impaired insulin secretion in response to sulfonylureas suggests that mitochondrial  $\text{Ca}^{2+}$  increases may be required to facilitate the ATP-dependent delivery of insulin granules to the plasma membrane.

*Supported by: JDRF, Wellcome Trust*

**Results:** All  $\beta$ -cells showed stable Perceval fluorescence in the presence of 1 mM glucose. Elevation of the glucose concentration to 3, 5, 7 and 9 mM dose-dependently increased the fluorescence intensity to a stable level with superimposed oscillations in less than 20% of the cells. Further glucose elevation to 11 and 20 mM had no effect on the average Perceval fluorescence but dramatically increased the number of oscillating cells, reaching 95% at the highest concentration. However, the ATP sensor was not saturated under these conditions since addition of 10 mM L-glutamine caused a 39% further increase of Perceval fluorescence. Although the amplitudes of the Perceval oscillations were unaffected, the frequency increased from  $0.23 \pm 0.01 \text{ min}^{-1}$  at 11 mM to  $0.31 \pm 0.01 \text{ min}^{-1}$  at 20 mM glucose. The appearance of Perceval oscillations clearly depended on  $\text{Ca}^{2+}$ . When  $\text{Ca}^{2+}$  influx was prevented by diazoxide hyperpolarization, the  $\text{Ca}^{2+}$  channel inhibitor methoxyverapamil or removal of extracellular  $\text{Ca}^{2+}$ , Perceval fluorescence immediately increased to a stable level without oscillations.

**Conclusion:** Sub-plasma membrane ATP levels in  $\beta$ -cells increase with the glucose concentration in the 0–9 mM concentration range. Higher glucose concentrations favour the appearance of oscillations without affecting the average ATP level. The oscillations depend on elevation of the cytoplasmic  $\text{Ca}^{2+}$  concentration, which lowers the sub-membrane ATP levels. The dynamic interplay between ATP and  $\text{Ca}^{2+}$  is important for the generation of metabolic oscillations and pulsatile insulin secretion.

*Supported by: Novo-Nordisk Foundation*

## 102

### Glucose-dependence of sub-plasma membrane ATP dynamics in pancreatic beta cells

J. Li, E. Gylfe, A. Tengholm;

Medical cell biology, Uppsala, Sweden.

**Background and aims:** ATP is an important messenger in  $\beta$ cell stimulus-secretion coupling by linking changes in glucose metabolism to electrical activity,  $\text{Ca}^{2+}$  signaling and insulin secretion. Measurements of oxygen consumption, NAD(P)H fluorescence and mitochondrial membrane potential indicate that the  $\beta$ cell metabolism oscillates. Yet there is little information about single-cell ATP dynamics and how glucose influences the ATP concentration of importance for insulin secretion in the sub-plasma membrane space.

**Materials and methods:** Islets from C57BL/6 mice were transduced with adenovirus expressing Perceval, an ATP sensor based on circularly permuted monomeric Venus fluorescent protein fused to the ATP-binding bacterial protein GlnK1. Fluorescence changes in the sub-plasma membrane space were recorded with total internal reflection fluorescence microscopy.

## OP 18 The -omics frontier: applications of new technologies

### 103

#### Enrichment of mRNA transcripts related to risk of type 2 diabetes in pathways involved in insulin resistance: KORA S4/F4 cohort

M. Carstensen<sup>1</sup>, C. Herder<sup>1</sup>, S. Landwehr<sup>2</sup>, K. Heim<sup>3</sup>, W. Rathmann<sup>2</sup>, B. Thorand<sup>4</sup>, C. Meisinger<sup>4</sup>, H.-E. Wichmann<sup>5</sup>, S. Martin<sup>6</sup>, H. Finner<sup>2</sup>, K. Strassburger<sup>2</sup>, T. Meitinger<sup>3,7</sup>, M. Roden<sup>1,8</sup>, T. Illig<sup>9,10</sup>, H. Prokisch<sup>3,7</sup>;

<sup>1</sup>Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf,

<sup>2</sup>Institute of Biometry and Epidemiology, German Diabetes Center,

Düsseldorf, <sup>3</sup>Institute of Human Genetics, Helmholtz Zentrum München,

Neuherberg, <sup>4</sup>Institute of Epidemiology II, Helmholtz Zentrum München,

Neuherberg, <sup>5</sup>Institute of Epidemiology I, Helmholtz Zentrum München,

Neuherberg, <sup>6</sup>West-German Centre of Diabetes and Health, Catholic

Hospital Group, Düsseldorf, <sup>7</sup>Institute of Human Genetics, Technical

University Munich, Munich, <sup>8</sup>Department of Metabolic Diseases, University

Hospital Düsseldorf, Düsseldorf, <sup>9</sup>Research Unit of Molecular Epidemiology,

Helmholtz Zentrum München, Neuherberg, <sup>10</sup>Hannover Unified Biobank,

Hannover Medical School, Hannover, Germany.

**Background and aims:** Biomarkers may lead to a better understanding of pathophysiological mechanisms in complex diseases. Gene expression profiles in various tissues are associated with type 2 diabetes in cross-sectional studies, but prospective transcriptomics studies for incident type 2 diabetes are lacking. The aim of this study was to identify biological pathways in which transcripts are enriched that are associated with incident type 2 diabetes.

**Materials and methods:** We investigated associations between genome-wide gene expression in peripheral blood and incident type 2 diabetes in 513 initially non-diabetic individuals within the population-based KORA S4/F4 cohort. During a follow-up of 7 years, 50 subjects developed type 2 diabetes. The association between mRNA transcripts and incident type 2 diabetes was assessed by logistic regression. We used Storey's critical values at a nominal false discovery rate of 0.1 for the correction for multiple testing. Gene-set enrichment analysis was performed using Ingenuity Pathway Analysis.

**Results:** After adjustment for age, sex and body mass index, 2,045 transcripts were found differentially expressed between incident cases and controls. Forty-three canonical pathways showed an enrichment of these differentially expressed transcripts. The strongest associations were observed for eIF2 (eukaryotic translation initiation factor-2) signalling, Benjamini-Hochberg adjusted  $P=2.93 \times 10^{-17}$ , eIF4/p70S6K (70-kDa ribosomal S6 kinase) signalling ( $P=9.19 \times 10^{-8}$ ) and mTOR (mammalian target of rapamycin) signalling ( $P=4.62 \times 10^{-7}$ ). We also found evidence ( $P$  between 0.05 and 0.0001) for an enrichment of differentially expressed transcripts and pathways involved in endoplasmic reticulum stress, inflammation, immune responses, lipid metabolism, endocrine function, mitochondrial function and cell/tumour proliferation and apoptosis.

**Conclusion:** In this first prospective transcriptomics study in incident type 2 diabetes we show that gene expression levels in peripheral blood are altered before the onset of type 2 diabetes and that differentially expressed transcripts are overrepresented in pathways related to cellular stress, regulation of protein synthesis and other processes which relate to insulin resistance.

**Supported by:** DZD, NGFNplus Atherogenomics (01GS0834), Leibniz Association (SAW)

### 104

#### MicroRNA expression fingerprint in serum of type 1 diabetic patients

G. Sebastiani<sup>1</sup>, I. Spagnuolo<sup>1</sup>, A. Patti<sup>1</sup>, F.A. Grieco<sup>1</sup>, D. Cataldo<sup>1</sup>, E. Ferretti<sup>2</sup>, C. Tiberti<sup>3</sup>, F. Dotta<sup>4</sup>;

<sup>1</sup>University of Siena and Umberto di Mario Foundation, <sup>2</sup>Dep. of

Experimental Medicine, University of Rome- Sapienza, <sup>3</sup>Dep. of Clinical

Sciences, University of Rome- Sapienza, Italy.

**Background and aims:** MicroRNAs (miRNAs) are a class of evolutionarily conserved small endogenous RNAs, 19–21 nucleotides long, which negatively regulate gene expression post-transcriptionally through their binding to the 3' untranslated region (3'UTR) of mRNA targets, thus affecting degradation and translation. MiRNAs are involved in many biological processes such as differentiation, cell cycle and apoptosis and their alterations have been observed in a number of pathological conditions, including autoimmune diseases. Recently,

it has been shown that miRNAs can be secreted by cells and their presence has been confirmed in many biological fluids including serum. In type 1 diabetes mellitus (T1D), the autoimmune process may be triggered years before clinical onset; indeed, it would be useful to identify novel and valid biomarkers for T1D prediction and follow-up, in addition to those already available (i.e. autoantibodies). Interestingly, a recent hypothesis focused on the possibility that circulating miRNAs may traffic from donor cells to distant sites and alter the gene expression of recipient cells. Indeed, any beta cell alterations of miRNA expression profile during T1D natural history may potentially affect the function(s) of cells receiving miRNA secreted from them. Consequently, the aim of our study was to look for a specific miRNAs fingerprint in T1D patient sera.

**Materials and methods:** To this end, we analyzed the expression profile of 384 miRNAs in serum of 20 newly-diagnosed T1D patients (age:  $31.3 \pm 10.8$  y) and 20 control subjects (age:  $32.2 \pm 8.3$  y), using stem-loop RT-PreAmp Real-time PCR and Taqman Low Density Array cards. All values were normalized using the external control ath-miR-159 and the endogenous controls snR-NAU6, miR-16 and miR-451. Array data was analyzed using the RQ Manager Software. Specific miRNAs expression data were subsequently validated using single assay RT Real Time PCR performed in duplicate.

**Results:** Among 384 miRNAs analyzed, 206 were detected in serum of the two groups, and among these, 64/206 were found differentially expressed between controls and newly-diagnosed T1D patients. Among these differentially expressed miRNA, we observed that 43/64 were downregulated and 21/64 upregulated compared to control subjects. By single assay RT Real-Time PCR, we confirmed that some miRNAs involved in immune processes (miR-155, miR-181a, miR-146a, miR-31, miR-199a) or involved in the regulation of the beta-cell function (miR-34a, miR-9), were indeed differentially expressed. Of note, we also observed that genes encoding for some differentially expressed miRNAs are located in the proximity of T1D genetic susceptibility regions (14q32.2 and 19p13.2) and therefore of particular interest.

**Conclusion:** In conclusion, these data demonstrate that some miRNAs are differentially expressed in newly-diagnosed T1D patients serum; Interestingly, some of these are involved in the regulation of immune response or of beta cell mass and function. Ongoing studies will further explore the role of these circulating miRNAs in order to establish whether they represent novel biomarkers for disease prediction and follow-up.

### 105

#### Epigenetic modifications in pancreatic islets from patients with type 2 diabetes

C.A. Ling<sup>1</sup>, T. Dayeh<sup>1</sup>, P. Volkov<sup>1</sup>, B. Yang<sup>1</sup>, A. Olsson<sup>1</sup>, E. Hall<sup>1</sup>, T. Rönn<sup>1</sup>, C. Wollheim<sup>2</sup>, M.D. Nitert<sup>3</sup>;

<sup>1</sup>Clinical Sciences, Lund University, Malmö, Sweden, <sup>2</sup>University Medical Center, Geneva, Switzerland, <sup>3</sup>University of Queensland, Brisbane, Australia.

**Background and aims:** Epigenetic factors, including DNA methylation and histone modifications, can help to explain how cells with identical DNA sequence can differentiate into different cell types with unique phenotypes. DNA methylation of CpG sites has been associated with transcriptional silencing. Recent studies from our group and others demonstrate that epigenetic modifications influence the pathogenesis of type 2 diabetes. We have shown that pancreatic islets from patients with type 2 diabetes exhibit increased DNA methylation and decreased expression of the insulin gene. Moreover, high levels of glucose increased DNA methylation of the insulin gene in clonal beta-cells. Other groups have shown that epigenetic modifications of *Pdx1* can decrease its expression and hence insulin secretion in rodent islets. The aim of the present study was to identify novel epigenetic modifications in pancreatic islets from patients with type 2 diabetes, using both a candidate gene approach and a genome-wide approach.

**Materials and methods:** DNA methylation was analyzed in human pancreatic islets from patients with type 2 diabetes and non-diabetic controls using both specific and genome-wide assays. These include EpiTYPER (Sequenom), Pyrosequencing (Qiagen) and the Infinium Human methylation 450k array (Illumina). DNA methylation was further related to islet gene expression and HbA1c levels.

**Results:** Pancreatic islets from patients with type 2 diabetes secrete less insulin in response to glucose in vitro compared with islets from non-diabetic donors ( $P < 0.05$ ). We identified individual CpG sites of the *PDX1* gene that exhibit increased DNA methylation in diabetic compared with non-diabetic human islets using a candidate gene approach ( $P = 8 \times 10^{-5}$ ). The degree of DNA methylation of these CpG sites correlated positively with HbA1c levels ( $\rho = 0.54$ ,  $P = 2.4 \times 10^{-5}$ ) and negatively with gene expression ( $\rho = -0.64$ ,  $P = 2.9 \times 10^{-5}$ ), suggesting that hyperglycaemia may increase DNA methylation



of *PDX1* in human islets. Using a genome-wide approach, a total of 3116 CpG sites were identified to be differentially methylated in pancreatic islets from patients with type 2 diabetes ( $Q < 0.05$  after a FDR analysis). Of these, 2988 CpG sites showed decreased and 128 CpG sites showed increased DNA methylation in islets from patients with type 2 diabetes. The identified CpG sites correspond to 1561 individual genes.

**Conclusion:** We have indentified genome-wide epigenetic modifications in pancreatic islets from patients with type 2 diabetes. Moreover, epigenetic modifications of *PDX1* were associated with reduced gene expression and impaired insulin secretion in human islets.

*Supported by: Swedish Research Council, ALF, EFSD/Lilly grant, Novo Nordisk foundation*

## 106

### Genome-wide methylation variations precede diagnosis of type 1 diabetes in children

N.A. West<sup>1</sup>, I.V. Yang<sup>2</sup>, T.E. Fingerlin<sup>1</sup>, G. Eisenbarth<sup>3</sup>, M. Rewers<sup>3</sup>, J.M. Norris<sup>1</sup>;

<sup>1</sup>Epidemiology, <sup>2</sup>Medicine, <sup>3</sup>Pediatrics, University of Colorado, Aurora, USA.

**Background and aims:** Epigenetic tagging of genes, such as DNA methylation, appears to be involved in autoimmune disease etiology. We aimed to interrogate the peripheral leukocyte genome for DNA methylation signatures associated with the development of type 1 diabetes (T1D).

**Materials and methods:** DNA was obtained prior to T1D diagnosis from 17 children younger than 10 years and from 17 age- and sex-matched non-diabetic, islet autoantibody negative controls followed by the Diabetes Autoimmunity Study in the Young (DAISY). Genome-wide DNA methylation profiles were determined using the comprehensive analysis of relative DNA methylation platform (CHARM) - a microarray-based method designed to examine 4.6 million CpG sites in the human genome using 2.1 million probes. Paired t-tests were used to identify differentially methylated CpG sites. A generalized moving average of p-values from hypothesis tests performed at each of the probes was calculated and corrected for false discovery rate.

**Results:** Using a pragmatic threshold of a corrected  $P < .05$ , we identified 16 differentially methylated regions (DMRs), associated with 16 genes, between cases and controls. Two of the identified genes, *SLC17A6* and *EIF4EBP2* (Table), have been associated with insulin and glucose regulation. *SLC17A6* encodes the glutamate transporter *VGLUT2*, which is expressed in both pancreatic  $\alpha$ - and  $\beta$ -cells and is involved in insulin and glucagon secretion, suggesting that this gene might play a significant role in diabetes pathogenesis. *EIF4EBP2* expression has been shown to be a component of the insulin signaling pathway with enhanced insulin signaling and increased glucose uptake as a consequence of reduced expression. Four of the identified genes have been specifically associated with development of T1D: *Siglec-15*, *RLTPR*, *SNTG1*, and *RXR $\alpha$*  (Table). Notably, the *SNTG1* gene has been associated with multiple sclerosis, a classical T cell-mediated autoimmune disease with a complex genetic background. Significant copy number variations within the *SNTG1* gene have also been reported between monozygotic twin pairs discordant for T1D. Further, differential DNA methylation within the *SNTG1* gene derived from purified CD14<sup>+</sup> monocytes was recently reported in a sample of T1D-discordant monozygotic pairs. The DMRs within the remaining genes (*GPR62*, *XKR4*, *FARP1*, *B4GALT3*, *PAQR9*, *ZNF679*, *MYL9*, *PTGFRN*, *LRRC4C*, and *OSBP*) have not been reported in T1D epigenome association studies. Thus, these regions may represent novel epigenetic risk loci for the disease.

**Conclusion:** Results from this study demonstrate different methylation patterns in T1D cases compared to age- and sex-matched controls in children with HLA-conferred genetic risk. Importantly, these patterns precede diagnosis of T1D in the cases, which suggest a possible etiologic role of T1D-associated epigenetic variation.

#### Differentially methylated genes between type 1 diabetes cases and controls

Gene Symbol	Chromosome	Mean % methylation cases/controls	Corrected P
<i>SLC17A6</i>	11	21.6/18.2	3.22 x 10 <sup>-3</sup>
<i>EIF4EBP2</i>	10	22.0/28.7	1.39 x 10 <sup>-2</sup>
<i>SIGLEC15</i>	18	28.5/36.6	1.44 x 10 <sup>-2</sup>
<i>RLTPR</i>	16	14.1/20.1	1.67 x 10 <sup>-2</sup>
<i>SNTG1</i>	8	22.1/18.1	1.89 x 10 <sup>-2</sup>
<i>RXRA</i>	9	24.0/21.5	3.06 x 10 <sup>-2</sup>

*Supported by: NIH R01-DK49654 and DK32493*

## 107

### Exercise induces altered DNA methylation in adipose tissue throughout the human genome

T.S. Rönn<sup>1</sup>, P. Volkov<sup>1</sup>, M. Dekker Nitert<sup>2</sup>, O. Hansson<sup>3</sup>, T. Elgzyri<sup>3</sup>, L. Groop<sup>3</sup>, K.-F. Eriksson<sup>4</sup>, C. Ling<sup>1</sup>;

<sup>1</sup>Clinical Sciences, Malmö, Epigenetics & Diabetes, Malmö, Sweden, <sup>2</sup>Royal Brisbane Clinical School, Herston, Australia, <sup>3</sup>Diabetes & Endocrinology, Malmö, <sup>4</sup>Vascular Diseases, Malmö, Sweden.

**Background and aims:** A sedentary lifestyle and poor diet makes obesity a growing health problem. Obesity is also a predictor for type 2 diabetes (T2D), which suggests a central role for adipose tissue in the pathogenesis of T2D, in combination with genetic and life-style factors. Physical exercise is important for weight maintenance, but also for improved insulin sensitivity. Recent studies point towards epigenetic changes to be involved in the regulation of genes important for glucose metabolism and the pathogenesis of T2D. Here we study genome-wide changes in DNA methylation in subcutaneous fat after an exercise intervention study, in healthy, but previously sedentary men.

**Materials and methods:** This study included 31 men from Malmö, Sweden, participating in a six months supervised exercise intervention study. At inclusion they were all sedentary but healthy, with a mean age of 37.4 years and a mean BMI of 27.8 kg/m<sup>2</sup>. Subcutaneous fat biopsies were obtained before and after the intervention, followed by DNA and RNA extraction. DNA methylation was analysed using Infinium HumanMethylation450 BeadChip assay (Illumina), covering 485577 CpG sites from 21231 genes. RNA microarray analysis was performed using GeneChip® Human Gene 1.0 ST array (Affymetrix).

**Results:** Complete DNA methylation data was obtained from 23 individuals both before and after the exercise intervention, including a total of 476753 probes after quality control. The average methylation was high in regions 200 bp upstream of transcription start site (TSS), in 5' untranslated regions (UTR) and in the first exon, and they all significantly increased after exercise ( $q \leq 0.01$ ). The average level of DNA methylation was low in regions 1500-200 bp upstream of TSS, within the gene body, in 3' UTRs and in intergenic regions, and did not show an overall change with exercise. Applying FDR correction ( $q < 0.05$ ) resulted in 17975 probes significantly different after exercise compared with baseline. We further filtered our results requiring the average change in DNA methylation ( $\beta$ -value) for each probe to be  $\geq 5\%$  between groups, which resulted in 1009 significant individual probes, 911 increasing and 98 decreasing, after the six months exercise intervention. Of these, 723 probes are located in relation to one or more genes, and correspond to 641 unique genes. One example is found in *ITPR2*, a locus reported to be associated with waist-hip ratio. Individual CpG-sites with a difference in methylation after versus before exercise ( $q < 0.05$  and difference in mean  $\beta$ -values  $\geq 5\%$ ) was matched with probe sets that changed in mRNA expression after exercise ( $q < 0.05$ ). Gene regions including 249 significant DNA methylation probes were found to also have a significant change in mRNA expression after exercise. Of these, 155 probes showed an inverse relation to mRNA expression. 151 probes showed an increase in DNA methylation and a corresponding decrease in gene expression, while only 4 probes decreased the level of DNA methylation together with increased gene expression.

**Conclusion:** We have shown a general increase in DNA methylation in human adipose tissue in response to long-term exercise, but also a change in DNA methylation on the level of individual probes and genes. Interestingly, 34% of the significant probes within gene regions also showed a difference in mRNA expression of that gene.

*Supported by: Nilsson,Thuring,Söderberg, Pahlsson Foundations SRC, EFSD/Lilly grant, ALF*

## 108

**Early metabolic markers of the development of dysglycaemia and type 2 diabetes and their physiological significance**

E. Ferrannini<sup>1</sup>, A. Natali<sup>2</sup>, S. Camastra<sup>3</sup>, M. Nannipieri<sup>3</sup>, A. Mari<sup>4</sup>, K.-P. Adam<sup>5</sup>, M. Milburn<sup>5</sup>, G. Kastenmuller<sup>6</sup>, J. Adamski<sup>7</sup>, T. Tuomi<sup>8</sup>, V. Lyssenko<sup>9</sup>, L. Groop<sup>9</sup>, W. Gall<sup>10</sup>, RISC Study Group;

<sup>1</sup>Internal Medicine, University of Pisa, Italy, <sup>2</sup>Clinical and Experimental Medicine, University of Pisa, Italy, <sup>3</sup>Medicine, University of Pisa, Italy, <sup>4</sup>Institute of Biomedical Engineering, National Research Council, Padova, Italy, <sup>5</sup>Metabolon, Inc., Durham, NC, USA, <sup>6</sup>Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany, <sup>7</sup>Institute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg, Germany, <sup>8</sup>Medicine, University of Helsinki, Finland, <sup>9</sup>Department of Clinical Sciences, Diabetes, and Endocrinology, Lund University, Malmö, Sweden, <sup>10</sup>Diagnostics, Metabolon, Inc., Durham, USA.

**Background and aims:** Metabolomic screening of fasting plasma from non-diabetic subjects identified alpha-hydroxybutyrate (AHB) and linoleoyl-glycerophosphocholine (L-GPC) as joint markers of insulin resistance (IR) and glucose intolerance. We tested their predictivity for incident dysglycemia and investigated their potential physiological role.

**Materials and methods:** Metabolite profiling was carried out in 1,261 non-diabetic participants of the RISC study and 2,580 subjects of the Botnia cohort. Three-year (RISC) and 9.5-year (Botnia) follow-up data were analyzed.

**Results:** In both RISC and Botnia baseline data, AHB was a positive correlate, and L-GPC a negative correlate, of insulin sensitivity; AHB was also reciprocally related to indices of beta cell function. In a subgroup of Botnia subjects, higher AHB was associated with higher branched-chain amino acid and free fatty acid levels, and decreased glycine (constituent of glutathione). In follow-up, AHB was a positive predictor (adjusted odds ratios 1.25 [95%CI:1.00-1.60] and 1.26 [95%CI:1.07-1.48], respectively) and L-GPC a negative predictor (adj. odds ratios 0.64 [95%CI:0.48-0.85] and 0.67 [95%CI: 0.54-0.84]) of dysglycemia (RISC) or type 2 diabetes (Botnia), independent of familial diabetes, sex, age, BMI, and fasting plasma glucose, with ROC area-under-curves of 0.791 and 0.783. In morbidly obese subjects undergoing bariatric surgery, AHB halved (6.14[3.64] to 3.47[1.43] microg/ml,  $p < 0.0001$ ) as insulin sensitivity doubled (19.9[17.4] to 41.4[10.5] micromol·min<sup>-1</sup>·kg<sub>ffm</sub><sup>-1</sup>,  $p < 0.0001$ ). Consistent with their association with insulin sensitivity and secretion, in INS-1E cell cultures, AHB inhibited and L-GPC stimulated glucose/arginine-induced insulin release at physiological concentrations.

**Conclusion:** AHB and L-GPC are early independent predictors of worsening glucose tolerance in diverse populations. Physiologically, AHB and L-GPC appear to be signatures of IR, beta cell dysfunction, and metabolic overload.

## OP 19 Novel therapies

## 109

**Optimising Phase 3 dose selection with ITCA 650: correlation between baseline HbA<sub>1c</sub> and reduction of HbA<sub>1c</sub> after 24 weeks of treatment**

R.R. Henry<sup>1</sup>, J. Rosenstock<sup>2</sup>, T. Alessi<sup>3</sup>, K. Luskey<sup>3</sup>, M. Baron<sup>3</sup>;

<sup>1</sup>University of California, San Diego, La Jolla, <sup>2</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, <sup>3</sup>Intarcia Therapeutics, Inc., Hayward, USA.

**Background and aims:** Current therapy with exenatide requires frequent injections and is associated with significant nausea, often leading to poor patient (pt) adherence with therapy that may compromise clinical outcomes. ITCA 650 is an injection-free form of exenatide therapy that provides continuous subcutaneous delivery for 3, 6 or 12 months from a single placement. Subcutaneous delivery of a stable daily dose of exenatide is expected to allow plasma levels to consistently reside within a predictable therapeutic window, thus providing effective glycemic control while mitigating side effects associated with variable exenatide plasma levels and ensuring pt adherence. ITCA 650 was evaluated in a 24-week phase 2 study to select the optimal dosing regimen for evaluation in phase 3 studies.

**Materials and methods:** Pts with type 2 diabetes (T2DM) (n=155) and mean baseline HbA<sub>1c</sub> of 8.0% were randomized to receive ITCA 650 20 mcg (n=51), or 40 mcg (n=51) or twice-daily injectable exenatide (5mcg titrated to 10mcg) (n=53) for a duration of 12 weeks (wks). After 12 wks, pts receiving ITCA 650 were re-randomized to continue treatment at their original dose or to increase to a higher dose: half of pts originally randomized to 20 mcg increased to 60 mcg, half of those receiving 40 mcg increased to 80 mcg and pts randomized to exenatide injections were assigned 1:1 to receive ITCA 650 40 mcg or 60 mcg. Therefore, approximately two-thirds of pts received ITCA 650 40 or 60 mcg during wks 13- 24 with the option to continue at that dose through wk 48.

**Results:** Overall, chronic administration of the higher doses of ITCA 650 (40, 60 and 80 mcg) had greater effects on reducing HbA<sub>1c</sub>, FPG, PPG and body weight compared to 20 mcg. The 40 and 60 mcg doses of ITCA 650 were better tolerated than 80 mcg. Further evaluation by baseline HbA<sub>1c</sub> revealed that substantially greater reductions in HbA<sub>1c</sub> were achieved in pts with higher baseline HbA<sub>1c</sub> treated with 60 mcg compared to 40 mcg (Table). Target HbA<sub>1c</sub> < 7.0% was achieved in 60% of pts treated with 60 mcg whose baseline HbA<sub>1c</sub> was > 8.0% with 44% achieving HbA<sub>1c</sub> ≤ 6.5%. By comparison 29% of pts on 40 mcg achieved HbA<sub>1c</sub> < 7.0% and none achieved ≤ 6.5%.

**Conclusion:** Based on recommended target goals to maintain HbA<sub>1c</sub> levels < 7.0% or ≤ 6.5%, this additional analysis of the ITCA 650 phase 2 study population by baseline HbA<sub>1c</sub> in combination with previously reported favorable tolerability and quality of life (QoL) profiles enhance the assessment of benefit risk and support the selection of a chronic dose of 60 mcg for phase 3 evaluation of ITCA 650.

Table 1 Change in HbA<sub>1c</sub> from Baseline and Proportion of Patients Reaching HbA<sub>1c</sub> Targets by Baseline

ITCA 650		HbA <sub>1c</sub> Change from Baseline			
Chronic Dose	n=	All	Baseline >7.0%	Baseline > 7.5%	Baseline > 8.0%
40 mcg/day	41	-0.86	-0.87	-0.90	-0.72
60 mcg/day	42	-1.38	-1.49	-1.77	-2.22
ITCA 650		Percentage of Patients with HbA <sub>1c</sub> < 7.0 at Week 24 by Baseline			
Chronic Dose	n=	All	Baseline >7.0%	Baseline > 7.5%	Baseline > 8.0%
40 mcg/day	41	69.0%	65.8%	55.6%	28.6%
60 mcg/day	42	70.7%	66.7%	63.0%	61.1%
ITCA 650		Percentage of Patients with HbA <sub>1c</sub> ≤ 6.5 at Week 24 by Baseline			
Chronic Dose	n=	All	Baseline >7.0%	Baseline > 7.5%	Baseline > 8.0%
40 mcg/day	41	33.3%	28.9%	18.5%	0.0%
60 mcg/day	42	48.8%	44.4%	40.7%	44.4%

## 110

**Effect of MK-3102, a novel once-weekly DPP-4 inhibitor, over 12 weeks in patients with type 2 diabetes mellitus**

I. Gantz, M. Chen, A. Mirza, S. Suryawanshi, M.J. Davies, B.J. Goldstein; Merck Sharp & Dohme Corp., Whitehouse Station, USA.

**Background and aims:** MK-3102 is a potent, oral, once-weekly, dipeptidyl peptidase-4 (DPP-4) inhibitor in development for the treatment of type 2 diabetes mellitus (T2DM). The convenience of an effective, well-tolerated, weekly oral drug has the potential to improve medication adherence and may translate into better long-term outcomes. The present study evaluated the 12-week efficacy and safety of MK-3102 monotherapy in patients with T2DM.

**Methods:** MK-3102 monotherapy was evaluated in a randomised, double-blind, placebo (PBO)-controlled, dose-range finding study. After a diet/exercise run-in and, for patients on an antihyperglycaemic agent, a drug wash-off period, eligible patients (N = 685) were randomised equally to one of six once-weekly (q.w.) treatments: PBO or MK-3102 0.25, 1, 3, 10, or 25 mg for 12 weeks. The primary efficacy endpoint was the change in HbA<sub>1c</sub> from baseline at Week 12. The analysis was based on a step-down trend test using a longitudinal model assuming the same baseline HbA<sub>1c</sub> with terms for treatment, prior antihyperglycaemic therapy status, geographic region, and the interaction of time by treatment and time by prior antihyperglycaemic therapy status.

**Results:** At baseline, randomised patients (56% males) had a mean HbA<sub>1c</sub> of 8.1%, mean fasting plasma glucose (FPG) of 9.5 mmol/L, and mean duration of diabetes of 5.4 years. At Week 12, MK-3102 significantly reduced HbA<sub>1c</sub> by 0.28% to 0.71% from baseline relative to PBO in a dose-dependent manner (Table). PBO-adjusted changes from baseline in 2-hour postmeal glucose (PMG) were significant at all doses and ranged from 1.0 to 2.5 mmol/L. Relative to PBO, FPG was significantly reduced from baseline with MK-3102 doses above 0.25 mg q.w. Treatment with MK-3102 was associated with a low incidence of hypoglycaemia (0 to 2%) that was similar to PBO (1%).

**Conclusion:** Treatment with once-weekly MK-3102 monotherapy improved glycaemic control and was generally well tolerated over 12 weeks in patients with T2DM.

Variable	Placebo	MK-3102 (mg q.w.)				
		0.25	1	3	10	25
Δ A1C, %	0.14 (-0.01, 0.29)	-0.14 (-0.30, 0.01) <sup>a</sup>	-0.36 (-0.51, -0.20) <sup>a</sup>	-0.35 (-0.50, -0.19) <sup>a</sup>	-0.53 (-0.68, -0.38) <sup>a</sup>	-0.57 (-0.73, -0.42) <sup>a</sup>
Δ 2-hr PMG, mmol/L	0.4 (-0.1, 1.0)	-0.6 (-1.2, -0.1) <sup>b</sup>	-1.4 (-2.0, -0.9) <sup>a</sup>	-1.5 (-2.1, -1.0) <sup>a</sup>	-1.9 (-2.4, -1.4) <sup>a</sup>	-2.1 (-2.6, -1.5) <sup>a</sup>
Δ FPG, mmol/L	0.2 (-0.1, 0.5)	0.1 (-0.3, 0.4)	-0.9 (-1.2, -0.5) <sup>a</sup>	-0.6 (-0.9, -0.3) <sup>a</sup>	-0.5 (-0.9, -0.2) <sup>a</sup>	-1.0 (-1.3, -0.7) <sup>a</sup>

n = 111–115/group; Data are expressed as LS mean change from baseline (95% confidence interval).

<sup>a</sup>p<0.001, <sup>b</sup>p<0.01, or <sup>c</sup>p<0.05 from trend test for MK-3102 dose vs. placebo.

Clinical Trial Registration Number: NCT01217073

Supported by: Merck Sharp & Dohme Corp.

## 111

**KRP-104, a DPP-4 inhibitor that uniquely matches extracellular target localisation with compound exposure, is a safe and effective candidate for type 2 diabetes**

T. Ide<sup>1</sup>, W. Hori<sup>2</sup>, J.S. Rosenblum<sup>3</sup>, L. Minimo<sup>3</sup>, T. Matsui<sup>1</sup>, Y. Kitamura<sup>1</sup>, T. Anraku<sup>2</sup>, K. Shimamoto<sup>2</sup>, M. Yamasaki<sup>2</sup>

<sup>1</sup>Discovery Research Laboratories, Kyorin Pharmaceutical Co., Ltd., Tochigi, Japan, <sup>2</sup>Development Research Laboratories, Kyorin Pharmaceutical Co., Ltd., Tochigi, Japan, <sup>3</sup>ActivX Biosciences, Inc., La Jolla, USA.

**Background and aims:** Dipeptidyl peptidase (DPP) IV is a validated drug target for diabetes. In general, selectivity is required for enzyme inhibitors to avoid adverse effects. *In vivo* selectivity is mainly determined by enzyme inhibition potency and drug concentration in relevant tissues. DPP-4 exists as both a membrane-anchored cell-surface protein and in the circulation. Therefore, there is no benefit to intracellular access for a DPP-4 inhibitor. Indeed, intracellular access for such compounds could be a serious liability. Here we show the unique pharmacodynamic, pharmacological and toxicological profile of KRP-104, a DPP-4 inhibitor that results in nearly exclusive extracellular compound exposure *in vivo*.

**Materials and methods:** Cell-free enzyme inhibition of KRP-104 and its metabolite GIS-103 were determined using substrate-based assays of human DPP-4-related enzymes. Cell permeability of GIS-103 was determined

by activity-based profiling of DPP-4 inhibition in intact cells treated with the compound. Tissue distribution studies of DPP-4 inhibitors were performed after single oral administration in rats.

**Results:** KRP-104 was rapidly converted *in vivo* into an active metabolite GIS-103. GIS-103 was a potent DPP-4 inhibitor (IC<sub>50</sub>: 3.4 nM) selective for DPP-4 over the related enzymes DPP-8 and DPP-9 (150-fold for DPP-8, 82-fold for DPP-9), completely inactive on cytosolic DPP-9 in cells treated with 20 μM compound (Fig.). GIS-103 was distributed almost exclusively to the extracellular compartment at therapeutic doses, due to low cellular membrane permeability at clinically relevant inhibitor concentrations. Furthermore, after a single oral dose of 10 mg/kg KRP-104 to fasted rats, the concentrations of GIS-103 in tissues were far lower than that of GIS-103 in plasma. In contrast, the DPP-4 inhibitors sitagliptin and vildagliptin were present in tissues at equal or even much higher concentrations than in plasma. GIS-103 stayed in the target organ (blood stream) and did not distribute to irrelevant tissues. Following oral administration to mice, KRP-104 and GIS-103 caused a dose related inhibition of plasma DPP-4 activity and a significant decrease in plasma glucose levels after oral glucose challenge. ICH-compliant toxicology programs and a 52-wk repeat-dose toxicity study in cynomolgus monkeys supported favorable safety profile.

**Conclusion:** The biological profile of KRP-104 is differentiated from other DPP-4 inhibitors and is characterized by *in vivo* selectivity. The combination of high selectivity for DPP-4, rapid and extensive conversion to GIS-103 *in vivo* after absorption, and extracellular distribution of GIS-103 may provide an advantage in avoiding adverse effects during long-term therapy for type 2 diabetes.

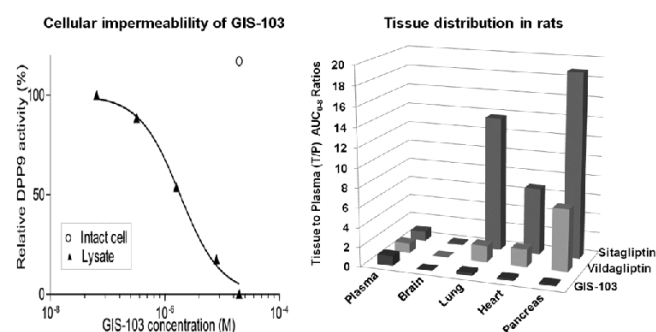


Fig. Cellular impermeability of GIS-103: intact HEK293 cells were treated with 20 μM GIS-103 concentration (circle). Following a 1 hour incubation, cells were washed, lysed and labeled with a serine hydrolase probe (AX7890). In parallel, lysate generated from HEK293 cells was treated with a gradient of GIS-103 and labeled with AX7890 (triangles). Tissue distribution in rats: tissue vs. plasma concentration (T/P) AUC<sub>0-24</sub> ratios after a single oral administration (10 mg/kg) in rats.

## 112

**12-week treatment with glucagon receptor antagonist LY2409021 significantly lowers HbA<sub>1c</sub> and is well tolerated in patients with type 2 diabetes mellitus**

C.M. Kazda<sup>1</sup>, P. Garhyan<sup>2</sup>, R.P. Kelly<sup>3</sup>, C. Shi<sup>4</sup>, C.N. Lim<sup>3</sup>, H. Fu<sup>2</sup>, M. Deeg<sup>2</sup>

<sup>1</sup>Medical department, Endocrinology, Eli Lilly and Company, Suresnes, France, <sup>2</sup>Eli Lilly and Company, Indianapolis, USA, <sup>3</sup>Eli Lilly and Company, Singapore, <sup>4</sup>Pharmanet/i3, Ellicott City, USA.

**Background and aims:** Type 2 diabetes mellitus (T2DM) pathophysiology is characterized by dysregulated glucagon secretion. LY2409021 (LY) is a potent, selective glucagon receptor antagonist that lowers glucose. This double-blind, Phase 2 study examined the margin between LY efficacy and safety by comparing mean changes in HbA<sub>1c</sub> and liver aminotransferases at 3 dose levels; other measures of glycemic control and safety were evaluated.

**Materials and methods:** Patients (pts) aged 18–70 with T2DM (HbA<sub>1c</sub> 6.5%–10%) naïve to antidiabetic medications or taking a stable metformin dose were randomized to LY 10 mg (n=17), 30 mg (n=34), 60 mg (n=26), or placebo (n=10) once a day for 12 weeks.

**Results:** At baseline, mean HbA<sub>1c</sub> for LY 10 mg (8.0%), 30 mg (7.5%), and 60 mg (7.6%) were similar to placebo (7.8%). At 12 weeks, HbA<sub>1c</sub> least-squares mean change from baseline showed all LY dosages improved glycemic control (10 mg: 0.83%, P=0.030; 30 mg: 0.65%, P=0.042; 60 mg: 0.66%, P=0.051) in contrast to placebo (0.11%). Secondary efficacy measures (eg, seven-point self-monitored blood glucose (SMBG) profile) supported superiority of LY over placebo. No significant changes in triglycerides, LDL- or HDL-cholesterol, weight, or blood pressure were observed. Dose-dependent increases in fasting glucagon, alanine aminotransferase (ALT), aspartate aminotrans-



ferase (AST), and total glucagon-like peptide-1 (GLP-1) were observed; these returned to baseline on washout of LY. Pts exposed at endpoint to LY 10 mg showed no significant ALT or AST changes; LY 30 and 60 mg were associated with mean increases ranging from 3.7 to 19.9 U/L. Total, direct, and indirect bilirubin, fasting insulin, and active GLP-1 were unchanged vs. placebo. Eight of 85 pts reported hypoglycemic events, 4 of which were confirmed. Incidence of hypoglycemia was not dose dependent; no severe events were reported. Adverse events frequency was similar with active treatment and placebo.

**Conclusion:** This study demonstrates that glucagon receptor antagonism with LY substantially lowers HbA<sub>1c</sub>. Increases in aminotransferases were evident at higher doses without elevated bilirubin or other signs or symptoms of liver injury. The efficacy, safety, and tolerability profile of LY in pts with T2DM supports further clinical development.

*Clinical Trial Registration Number:* NCT00871572

*Supported by:* Eli Lilly and Company

## 113

### Orally active small molecule AdipoRs agonists reduce metabolic stress, leading to anti-aging such as increased mitochondria and amelioration of obesity-related disorders

T. Yamauchi, M. Okada-Iwabu, M. Iwabu, T. Kadowaki;

Department of Metabolic Diseases, University of Tokyo, Japan.

**Background and aims:** Adiponectin secreted from adipocytes has been reported to mimic the effects of exercise via AdipoR1 in muscle. Ca<sup>2+</sup> signaling as well as AMPK and PGC-1 $\alpha$  in muscle are key mediators activated by both exercise and adiponectin. Importantly, in liver, adiponectin activates not only AMPK pathways via AdipoR1 but also PPAR $\alpha$  pathways via AdipoR2, leading to reduced ectopic fat accumulation. Thus, orally active small molecules that could bind to and activate both AdipoR1 and AdipoR2 have been expected to produce a metabolic profile desirable for treating diseases of aging such as type 2 diabetes and atherosclerosis. However, no paper has been published identifying such compounds.

**Materials and methods:** Here we describe the identification of orally active small molecules that could be AdipoR agonists.

**Results:** One of these compounds, AdipoR agonist (ARA)-1, bound to both AdipoR1 and AdipoR2 *in vitro*, and activated AMPK, increased PGC-1 $\alpha$  levels in a Ca<sup>2+</sup> dependent manner and increased the number of mitochondria in myocytes. Orally administered ARA-1 ameliorated insulin resistance and glucose intolerance (insulin resistance index: 100 $\pm$ 9 vs. 57 $\pm$ 4%, vehicle vs. ARA-1,  $P < 0.01$ ) in control, but not in AdipoR1/AdipoR2 double knockout mice fed a high-fat diet. In livers of obese diabetic mice, ARA-1 suppressed molecules involved in gluconeogenesis via AdipoR1, increased PPAR $\alpha$  target genes involved in fatty-acid combustion and reduced oxidative stress via AdipoR2. In muscles, ARA-1 via AdipoR1 increased mitochondrial biogenesis, which was associated with increased exercise endurance, and at the same time increased levels of molecules involved in fatty-acid combustion and in the reduction of oxidative stress. In white adipose tissues (WAT), ARA-1 reduced oxidative stress, the production of pro-inflammatory cytokines, and the accumulation of M1 macrophages. In brown adipose tissues, ARA-1 increased the levels of molecules involved in energy dissipation. Importantly, these effects resulted in reduced tissue triglyceride content and oxidative stress in liver, muscle, and WAT and decreased inflammation in liver and WAT.

**Conclusion:** Thus, small molecule AdipoR agonists are promising novel oral therapies for treating diseases of aging such as type 2 diabetes.

## 114

### Oral salmon calcitonin attenuates hyperglycaemia and preserves pancreatic beta cell mass and function in Zucker diabetic fatty rats

M. Feigh<sup>1</sup>, K. Vietz Andreassen<sup>1</sup>, A.V. Neutzsky-Wulff<sup>1</sup>, C. Hansen<sup>1</sup>, S.T. Petersen<sup>1</sup>, J. Henriksen<sup>2</sup>, H. Beck-Nielsen<sup>2</sup>, C. Christiansen<sup>1</sup>, K. Henriksen<sup>1</sup>, M.A. Karsdal<sup>1</sup>;

<sup>1</sup>Nordic Bioscience, Herlev, <sup>2</sup>Department of Endocrinology M, Odense University Hospital, Odense, Denmark.

**Background and aims:** Oral delivery of the peptide hormone salmon Calcitonin (sCT) possesses glucoregulatory effects in diet-induced obese and insulin resistant rats. In here, we describe a proof of concept study in the Zucker diabetic fatty (ZDF) rat, a model of type 2 diabetes, investigating the effects

of oral sCT on diabetic hyperglycemia and exploring the mode of actions by which it may protect against type 2 diabetes.

**Materials and methods:** Male ZDF rats were treated with oral sCT (0.5, 1.0 or 2 mg/kg) or oral vehicle twice daily from age 8 to 18 weeks. Zucker lean rats served as control group. Fasting and non-fasted blood glucose, HbA<sub>1c</sub> and levels of pancreatic and incretin hormones were determined. OGTT and i.p. glucose tolerance test (IPGTT) were compared and beta-cell mass and function evaluated.

**Results:** Oral sCT treatment dose-dependently attenuated fasting and non-fasted hyperglycaemia during the intervention period. At the end of the study period, oral sCT treatment by dose decreased plasma glucose levels by ~9 mM and reduced HbA<sub>1c</sub> levels by 1.7% compared to vehicle. Furthermore, compared to vehicle, a marked ~60% reduction in glucose excursions was dose-dependently observed for oral sCT treatment during OGTT, although much less pronounced during IPGTT. During the intervention period, in contrast to vehicle, oral sCT treatment sustained hyperinsulinemia, ameliorated hyperglucagonemia and attenuated hypersecretion of the incretin hormone glucagon-like peptide-1. Lastly, oral sCT treatment dose-dependently improved pancreatic beta-cell function and beta-cell area at study end.

**Conclusion:** Oral salmon Calcitonin attenuated diabetic hyperglycemia in ZDF rats by improving postprandial glycemic control, exerting an insulinotropic and glucagonostatic action and by preserving pancreatic beta-cell mass and function.

*Supported by:* Den Danske Forskningsfond

## OP 20 Insulin: beyond traditional delivery

115

### Buccal spray insulin in subjects with impaired glucose tolerance: improvement in HbA<sub>1c</sub> is lost after 6 months wash out therapy

A. Palermo<sup>1</sup>, N. Napoli<sup>1</sup>, E. Maddaloni<sup>1</sup>, A. Lauria<sup>1</sup>, A. Soare<sup>1</sup>, S. Manfrini<sup>1</sup>, M. Altomare<sup>2</sup>, S. Leotta<sup>2</sup>, P. Pozzilli<sup>1</sup><sup>1</sup>University campus Bio medico, <sup>2</sup>Hospital "S.Pertini", Roma, Italy.

**Background and aims:** subjects who develop type 2 diabetes (TD2) pass through a phase of impaired glucose tolerance (IGT). Defects in the action or secretion of insulin are the two major abnormalities leading to development of glucose intolerance. Resistance to insulin progressively increases when passing from normal glucose tolerance through IGT to diabetes, whereas secretion of insulin gradually decreases. Glucose tolerance is assumed to remain normal as long as the beta cell secretion can compensate for insulin resistance. IGT will develop only when insulin secretion fails to compensate fully for such resistance, resulting in postprandial hyperglycaemia that is linked to an increased risk for cardiovascular disease even though there is no progression to diabetes. Many intervention trials have been conducted in IGT patients but only one aimed to decrease post prandial hyperglycaemia. Our previous proof of concept study in subjects with IGT undergoing a prolonged OGTT demonstrated that treatment with 12 puffs of buccal spray insulin was followed by a significant 29.6% decrease in mean plasma glucose at two-hours and a 26.8% decrease at three-hours. Based on these findings a short trial with buccal spray insulin was planned.

**Materials and methods:** We have designed a randomized controlled trial in patients with IGT comparing a 6 months duration therapy using buccal spray insulin (12 puffs per meal) plus physical exercise and diet (treatment group A, n=16, HbA<sub>1c</sub> at entry 6.2% + 0.4) vs physical exercise and diet only (control group B, n=16, HbA<sub>1c</sub> at entry 6.0% + 0.3). Primary endpoint of this study is the reduction of HbA<sub>1c</sub> of 0.3 % at 6 month treatment between experimental versus control group. Secondary endpoints include the evaluation of antibodies against insulin (IA), changes in body weight, number of hypoglycaemic events during the treatment period and the evaluation of metabolic control at 6 months after the end of the treatment. HbA<sub>1c</sub> levels, metabolic parameters and insulin antibodies were measured at baseline, at 3 months up to 6 months followed by 6 months wash out.

**Results:** Subjects treated with buccal spray insulin achieved a significant reduction of HbA<sub>1c</sub> compared to the control group ( $\Delta$  HbA<sub>1c</sub> 0<sup>-</sup> 6 months -0.3% vs +0.09% p=0.002). At 6 months after the end of treatment, in group A HbA<sub>1c</sub> levels raised from 5.8 % + 0.3 to 6.1 + 0.5 resulting in loss of previous achieved improvement of metabolic control and 19% of the treated patients developed TD2 (vs 6% in the control group). There was no significant difference in body weight and no hypoglycaemic or other adverse events were observed during the study period in both groups. No generation of IA was observed in subjects with IGT treated with buccal spray insulin.

**Conclusion:** These results indicate that buccal spray insulin is an effective treatment compared to diet + physical exercise in patients with IGT in reducing HbA<sub>1c</sub> without adverse effects. However the beneficial effects of buccal spray insulin is lost within few months of suspension of treatment. A larger trial is required to demonstrate the long term effects of this buccal spray insulin in preventing TD2 in subjects with IGT.

Supported by: Educational grant from Generex Biotechnology

116

### Increase in microcirculatory perfusion after insulin treatment in poorly controlled type 2 diabetic patients: the INSULin regimens and VAScular functions (INSUVASC) study

M. Fysekidis, K. Takbou, Y. Jaber, E. Cosson, P. Valensi;

Diabetes, Endocrinology and Human Nutrition Department, Jean Verdier Hospital, APHP, Bondy, France

**Background and aims:** Insulin induces peripheral vasodilation in healthy subjects. The activity of the autonomic nervous system affects peripheral microcirculation and may be changed by insulin treatment. The long term effect of the improvement of blood glucose control obtained by insulin on microcirculation has not been studied in type 2 diabetic patients. The aim of this study was to examine the changes in cutaneous blood flow (CBF) in poorly

controlled type 2 diabetic patients at fasting and after a standard breakfast, before and after insulin treatment, and the respective role of the improvement of blood glucose control at fasting, post-prandially or both.

**Materials and methods:** The INSUVASC study is a unicenter pilot, randomised, open label study. We included 42 poorly controlled type 2 diabetic patients. CBF was measured during 15 minute periods with a Laser Doppler device (Periflux System 5000\*) at fasting (H0) and one and two hours (H1, H2) after a standardised breakfast that provided 75gr of carbohydrates. Variations in CBF during each period were analysed by spectral analysis for peaks at 0.1 Hz frequency known to represent with sympathetic activity. Measurements were performed before and after a 4-week randomised treatment with three different insulin regimens (Aspart, Detemir or Aspart combined with Detemir).

**Results:** A total of 34 patients completed the study and had good quality Laser Doppler measurements (Aspart, n=8, Detemir, n= 14 or Aspart combined with Detemir, n=12). Age, Body Mass Index, HbA<sub>1c</sub> and fructosamine at baseline did not differ significantly among groups, there were more men in the Detemir group (p= 0.003). CBF changes were expressed as percentages of the first baseline value. CBF increased after breakfast, before (H2: 157±77%, p< 0.001) and after (H2: 185±40%, p=0.005) four weeks of insulin treatment. CBF at fasting increased significantly after insulin treatment (H0: 130 ± 61%, p=0.007), with a similar trend for H1 (p=0.06) but not for H2. Blood flow measured in arbitrary perfusion units after insulin treatment during H0 (p=0,017), H1 (p=0,004) and H2 (p=0,048) correlated with the number of prescribed insulin units / kg of body weight. There were no significant correlations for the same periods before insulin treatment. Spectral analysis showed no significant change for the amplitude of the 0.1 Hz peak. There was no significant difference for the changes in CBF between the three insulin regimens.

**Conclusion:** Cutaneous blood flow increase after meal. The augmentation of CBF at fasting after insulin treatment although may be partly due to the improvement of blood glucose control itself, remains also consistent with the vasodilatory effect of insulin. The absence of changes in sympathetic activity, as shown from the absence of increase of the low frequency peak, suggests that other mechanisms like improved rheological factors, endothelial or myogenic activity may be involved.

Clinical Trial Registration Number: NCT01022658

Supported by: Novo Nordisk

117

### Insulin degludec does not increase antibody formation compared to insulin glargine: an evaluation of phase 3a clinical trials

J. Vora<sup>1</sup>, P. Hollander<sup>2</sup>, S.C. Tamer<sup>3</sup>, T. Johansen<sup>3</sup>, R. Bergenstal<sup>4</sup>;<sup>1</sup>Royal Liverpool University Hospitals, UK, <sup>2</sup>Baylor University Medical Center, Dallas, USA, <sup>3</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>4</sup>International Diabetes Center at Park Nicollet, Minneapolis, USA.

**Background and aims:** Insulin degludec (IDeg) is an ultra-long-acting basal insulin analogue where the threonine in position B30 of human insulin has been omitted and the lysine in position B29 has been coupled to hexadecanedioic acid via a glutamic acid spacer. IDeg forms soluble multi-hexamers upon s.c. injection, resulting in a stable and consistent glucose-lowering effect. All insulin preparations cause some degree of antibody formation in humans, but this has little or no effect on the efficacy or safety of currently marketed products. Here we present antibody data from all phase 3a clinical trials in patients with type 1 or type 2 diabetes where insulin antibody development was analysed for IDeg and compared to insulin glargine (IGlar).

**Materials and methods:** Insulin antibodies were measured in six randomised, controlled, open-label, treat-to-target trials (26 – 52 weeks' duration): two trials in type 1 diabetes (IDeg: n=801; IGlar: n=315) and four trials in type 2 diabetes (IDeg: n=1734; IGlar: n=860). In all trials, IDeg and IGlar were dosed once daily. Insulin aspart was used as bolus insulin in the type 1 diabetes trials. Antibody levels (specific and cross-reacting to human insulin) were measured using specific radioimmunoassays and expressed as percent bound/total radioactivity (% B/T).

**Results:** Across all trials, very few patients developed IDeg- and IGlar -specific antibodies and only at very low levels. Furthermore, mean levels of antibodies cross-reacting to human insulin remained low for both groups throughout the duration of the trials for subjects with type 1 diabetes (10 – 15% B/T) and type 2 diabetes (<6% B/T). For both IDeg and IGlar, Spearman correlation coefficients were calculated on an individual trial basis to investigate the influence of cross-reacting antibodies on HbA<sub>1c</sub>, change from baseline in HbA<sub>1c</sub>, and total daily insulin dose at end-of-trial. All correlation

coefficients were low indicating that there was no clinically relevant influence of insulin antibodies on  $HbA_{1c}$  or dose. Based on scatter plots, there was no difference in  $HbA_{1c}$  or total insulin dose at end-of-trial between patients having cross-reacting antibody levels of >10% B/T and those with levels of <10% B/T. The adverse-event profile in patients with an antibody titre of >10% B/T was generally similar to that of the overall trial population.

**Conclusion:** The immunogenic responses to long-term treatment with IDeg and IGLar are similar and low, with no clinically relevant impact on  $HbA_{1c}$  or total daily insulin dose.

Supported by: Novo Nordisk

## 118

### Reduced postprandial glucose excursion in type 2 diabetic subjects using the InsuPad device

G. Bitton<sup>1</sup>, I. Raz<sup>2</sup>, A. Pfutzner<sup>3</sup>, D. Feldman<sup>1</sup>, T. Alon<sup>1</sup>, L. Liviatan<sup>1</sup>, R. Nagar<sup>1</sup>;

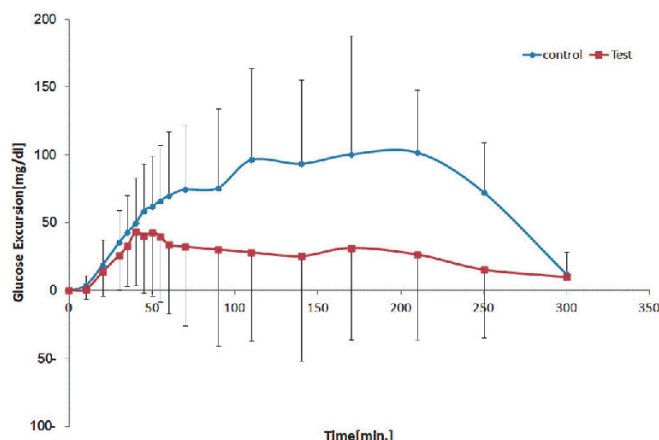
<sup>1</sup>Insuline Medical Ltd., Petach Tikva, Israel, <sup>2</sup>The Diabetes Center, Hadassah Hebrew University School of Medicine, Jerusalem, Israel, <sup>3</sup>IKFE–Institute for Clinical Research and Development, Mainz, Germany.

**Objective:** The pharmacodynamics (PD) and pharmacokinetics (PK) profiles of current insulin analogs are still slow compared to normal physiology. Among other effects this results in large post prandial blood glucose excursions in insulin dependent diabetic subjects (IDDS). InsuLine Medical has developed a technology to accelerate insulin PK profile by applying local controlled heat to the insulin delivery site. This heat increases local blood flow which can induce faster insulin clearance from the injection site which can lead to a better glycemic control. In this study we have tested the effect of the InsuPad device on post prandial glucose during meal tolerance test study.

**Methods:** A meal tolerance test protocol was conducted in type II diabetic subjects using insulin injections with rapid acting insulin analogs. Subjects injected 0.2U/kg before meal and consumed standardized liquid meal (Ensure Plus, Abbott, USA, 474 ml, carbohydrate 100g, protein 26 gm, fat 23 gm). Blood samples for glucose and insulin measurements were taken from a venous line during the whole study. The study was repeated twice with (test) and without (control) the use of the InsuPad device.

**Results:** 10 type II diabetic subjects participated in the study, aged 51 to 68 years [mean - 61.4 years], with  $HbA_{1c}$  of 7% to 9.8% [mean - 8.1%], BMI in the range of 23–35 [mean - 26.5 kg/m<sup>2</sup>] and mean diabetes duration of 24.5 years. The figure below shows the post meal glucose excursion with and without the use of the InsuPad device. Glucose excursion during 250 minutes after meal start ( $G-AUC_{0-250}$ ) was found to decrease when the InsuPad device was used ( $23.9 \pm 43.4$  mg/dL/H with the device vs  $63 \pm 36$  mg/dL/H without the device  $p=0.08$ ), glucose excursion at 120 minutes post meal ( $G_{120}$ ) was found to decrease when the InsuPad device was used ( $28 \pm 65.3$  mg/dl with the device vs  $96.4 \pm 67.5$  mg/dl without the device  $p=0.033$ ) Maximal glucose excursion ( $G_{max}$ ) was also decreased when the InsuPad device was used ( $79.8 \pm 52.2$  mg/dl vs  $136.8 \pm 53.6$  mg/dl  $p=0.053$ )

**Discussion and conclusion:** The above results show a mean reduction of more than 60% in post prandial glucose excursion during 6 hours after meal start. Such a significant decrease if repeated in daily life conditions can significantly improve glycemic control and reduce  $HbA_{1c}$  levels in type II diabetic subjects. Future plans are to increase the number of subjects participating in the study and repeat the study in daily life conditions. Figure 1. Post meal glucose excursion with and without the InsuPad device.



## 119

### Improved pharmacokinetic and pharmacodynamic profile of insulin analogues using InsuPatch, a local heating device

Z. Landau<sup>1</sup>, D. Feldman<sup>1</sup>, A. Shusterman<sup>1</sup>, J. Wainstein<sup>1</sup>, D. Klonoff<sup>2</sup>, I.

Nyberg<sup>2</sup>, D. Lender<sup>3</sup>, O. Mosenzon<sup>3</sup>, I. Raz<sup>3</sup>;

<sup>1</sup>Diabetes Unit, E. Wolfson Medical Center, Holon, Israel, <sup>2</sup>Diabetes Research Institute, Mills-Peninsula Health Services, San Mateo, USA, <sup>3</sup>Diabetes Unit, Hadassah University Hospital, Jerusalem, Israel.

**Background and aims:** The pharmacological profile of rapid acting insulin analogs is still far from mimicking the profile of endogenous insulin release. Studies suggest that heating the insulin injection site may accelerate insulin absorption by increasing local blood flow. InsuPatch is a local heating device intended to accelerate subcutaneous insulin absorption and activity while using an insulin pump, by locally warming the infusion site. The device warms the tissue surrounding the infusion site to 38.5 °C for 30 minutes following the administration of an insulin bolus. The aim of this study was to compare the pharmacodynamic and pharmacokinetic profile of subcutaneous administration of rapid-acting insulin analogs by insulin pump with and without InsuPatch.

**Materials and methods:** Euglycemic glucose clamp tests were performed in 56 subjects with type 1 diabetes, insulin pump users (mean age 33.4±13.5 years, 23 female,  $HbA_{1c}$  7.75±0.85%) after administration of 0.15 units/kg body wt of short-acting insulin analogs, with or without InsuPatch, using a randomized, cross over design. Fifty-five subjects completed the study. Each subject had three clamp procedures: two clamp studies with the InsuPatch device on Day 1 and on Day 3 of a 3-day infusion set cycle and one clamp study without the device on Day 1 of the infusion set cycle (Control). The second clamp study with InsuPatch on Day 3 was undertaken to examine whether there was any effect of increasing duration of infusion site use on the absorption and time action profile of short-acting insulin analogs.

**Results:** The area under the insulin concentration curve during the initial 60 minutes (insulin AUC0-60) was found to increase by 29.7±7% with InsuPatch use compare to control on Day 1 ( $P=0.012$ ). The insulin AUC0-60 was found to increase by 27.9±7% on Day 3 with InsuPatch compare to Day 1 with InsuPatch ( $p<0.05$ ). The table below lists the results for days 1 and 3 under test and control conditions for maximal insulin concentration (C-INSmax), Time to maximum glucose infusion rate (T-GIRmax), and maximal glucose infusion rate (GIRmax). The effect of the device was found to be higher for the subgroup of subjects with  $HbA_{1c}$  above the mean  $HbA_{1c}$  (7.76%,  $n=26$ ); the relative increase in AUC0-60 was 45% ( $p<0.05$ ). The same incidences of local irritations with and without the heating device (on Day 1 and on Day 3) were documented.

**Conclusion:** Administration of short acting insulin analogs to insulin pump treated subjects with type 1 diabetes with InsuPatch, a local heating device, enhances insulin absorption and reduces the duration of glucose-lowering action. This profile may help to achieve better meal insulin coverage and by this mean will permit better control of post-prandial glycemia.

Pharmacological parameters for insulin administration with and without InsuPatch

	InsuPatch Day 1	InsuPatch Day 3	Control Day 1	P; InsuPatch Day 1 vs. Control Day 1	P; InsuPatch Day 1 vs. InsuPatch Day 3
C-INS max [mU/L]	57	69	47	<0.05	<0.05
T-GIR max [min.]	93	78	124	<0.05	0.12
GIR max [mg/kg/min]	5.5	6.5	4.8	0.16	0.08

Clinical Trial Registration Number: NCT01216618



## 120

**In vitro characterisation of novel basal insulin LY2605541: reduced mitogenicity and IGF-IR binding**

R.A. Owens, J.F. Lockwood, J.D. Dunbar, C. Zhang, X. Ruan, S.D. Kahl, H.-R. Qian, J.M. Beals;  
Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, USA.

**Background and aims:** LY2605541 (LY) is insulin lispro with a 20kDa polyethylene glycol (PEG) moiety covalently attached to lysine B28. It is designed to have a large hydrodynamic diameter to reduce subcutaneous absorption and plasma clearance, which should provide a prolonged duration of action that is desired for a basal insulin. Given this design, it is critical to understand the in vitro biological properties of LY in terms of function, selectivity, and mitogenicity.

**Materials and methods:** Zinc-free biosynthetic human insulin, insulin lispro AspB10 insulin, and LY were dissolved in PBS, pH 7.4. Insulin detemir and insulin glargine were purchased, dissolved in diluents, and treated with Chelex 100 resin to remove zinc from the formulations, then dialyzed into PBS or citrate buffer, pH4, respectively. 293HEK cell membranes over-expressing either the human insulin receptor (IR) or the human insulin-like growth factor-1 receptor (IGF-1R) were used in a competitive radioligand scintillation proximity binding assay (SPA) in albumin-free NP-40 binding buffer. A tyrosine phosphorylation ELISA was used to determine the functional activity in IR over-expressing cells. Additional analysis included de novo lipogenesis from [ $^{14}$ C]-Glucose using differentiated 3T3-L1 adipocytes. Since insulin and IGF-1 act through their cognate receptors to increase DNA synthesis and cell proliferation, 2 cell lines—a human osteosarcoma cell line (SAOS2) that expresses more IGF-1R than IR and a rat hepatoma cell line (H4IIE) that expresses only rat IR—provide cellular models for in vitro mitogenicity as determined by methyl- $^3$ H-Thymidine incorporation into the cellular DNA of SAOS-2 cells, and proliferation (Cell Titer, Promega) in the H4IIE cells. Data is analyzed using four-parameter nonlinear regression (Activity Base, IDBS). Results are reported as the dose required to achieve a half-maximal response ( $EC_{50}$ ) compared to the BHI reference standard. The affinity constant ( $K_i$ ) was calculated from the  $IC_{50}$  value using the Cheng-Prusoff equation. All results are the geometric mean  $\pm$  SEM, n.

**Results:** LY shows 17-fold lower binding affinity than insulin lispro at IR ( $7.1 \pm 2.5$  nM; n=3 and  $0.41 \pm 0.02$  nM; n=3, respectively) and >32-fold lower binding affinity than insulin lispro at IGF-1R ( $>1500$  nM; n=3 and  $48 \pm 4$  nM; n=3, respectively). Based on these receptor binding results, LY is more selective than insulin lispro for IR v. IGF-1R ( $>210$ -fold v. 110-fold, respectively). LY is a fully efficacious and potent agonist for IR auto-phosphorylation ( $EC_{50}=20 \pm 2$  nM; n=3) and de novo lipogenesis ( $EC_{50}=16 \pm 4$  nM; n=6). LY shows reduced mitogenic potency compared to insulin lispro in both the IGF-1R-dominant and the IR-dominant cell line (78-fold less and 15-fold less, respectively). In comparison to other insulins, LY shows similar in vitro characteristics for signaling mediated through IR as compared to insulin lispro, insulin detemir, and insulin glargine. LY shows improved selectivity for IGF-1R signaling as compared to insulin lispro and insulin glargine.

**Conclusion:** Even with the reduced binding affinity following PEGylation, LY maintained all of the in vitro characteristics of its parent, insulin lispro, and achieved all of the metabolic and mitogenic characteristics desired in a long-acting insulin.

Supported by: Eli Lilly and Company

## OP 21 Stem cells in chronic complications

## 121

**Impact of IAPP on the cross-talk between pancreatic endothelial cells and pancreatic islet cells**

M. Visa<sup>1,2</sup>, M. Soty<sup>1,2</sup>, L. Cadavez<sup>1,2</sup>, J.M. Servitja<sup>1,2</sup>, A. Novials<sup>1,2</sup>;  
<sup>1</sup>Diabetes and Obesity Laboratory, IDIBAPS, Hospital Clinic, <sup>2</sup>CIBERDEM, Barcelona, Spain.

**Background and aims:** The close proximity of endothelial cells (EC) and beta-cells suggests that there is a cross-talk between these both cell types. EC produce molecules that could directly influence beta-cell function, such as collagen IV and laminins, which potentiates insulin secretion and proliferation. Nitric oxide (NO) produced by EC also influence beta-cell insulin release. IAPP is a beta-cell secreted peptide which seems to have some effects on endothelial function. Indeed, it has been implicated in vascular tone through a direct interference on NO release by the endothelium. Moreover, preliminary data from our group indicate that treatment of HUVEC cells with IAPP induces expression of VCAM. Taken together, these data suggest that IAPP could play a role on the islet endothelium and therefore affect beta-cell function and proliferation. The main objective of this work is to investigate the effect of IAPP on the cross-talk between pancreatic EC and pancreatic islet cells.

**Materials and methods:** Mouse pancreatic islets were placed on a collagen gel to obtain EC. After one week, EC were recovered and maintained in culture until passage number 8-10. Von Willebrand factor (vWF) and CD31 staining were used to characterize the EC. To analyze the effect of IAPP on EC, we treated EC at several doses of IAPP (10pM-100pM-1000pM) in a time-dependent manner. We measured pAkt/Akt and peNOS/eNOS ratio by western blot, and gene expression of adhesion molecules, ICAM and MCP-1, by real-time PCR. The proliferative capacity was analyzed by PCNA protein expression and BrdU incorporation. To further investigate the effect of IAPP on the cross-talk between pancreatic EC and beta-cells, islets were disaggregated by trypsin treatment to obtain islet cells (DIC). DIC were cultured in the presence or absence of EC treated or untreated with IAPP. The proliferative capacity of islet cells was determined by staining of the Ki67 marker.

**Results:** Western blot analysis showed that IAPP induced phosphorylation of Akt and eNOS in a dose-dependent manner. In addition, IAPP increased PCNA expression and BrdU incorporation. This increase was more important when EC was treated at 100pM IAPP for 48h (PCNA expression: 2.5 fold over untreated cells  $p<0.05$ ; BrdU incorporation: 57.2% compared to untreated cells  $p<0.05$ ). These results show that IAPP stimulates the phosphorylation of Akt and eNOS and that the activation of these signaling molecules mediates the effect of IAPP on EC proliferation capacity. Moreover, EC treated with IAPP showed an increase in the expression of genes related to cell adhesion such as MCP-1 (2.5 fold,  $p<0.05$ ) and ICAM (2.3 fold,  $p<0.05$ ) relative to untreated cells. The co-culture of DIC with an EC basement induced reorganization in cell clusters of DIC, expressing insulin and glucagon. The presence of EC allowed the proliferation of islet cells, which was demonstrated by Ki67 immunolabelling in the nucleus of glucagon and insulin-marked cells. In addition, the co-culture treated with IAPP showed higher proliferative capacity compared to untreated co-culture.

**Conclusion:** We suggest that IAPP induces the proliferative capacity of EC through the activation of the Akt-eNOS pathway and increases the expression of cell adhesion molecules. These effects improve the proliferative capacity of islet cells cultured in the presence of EC.

Supported by: FIS (PI08/0088) and (PI11/00679)

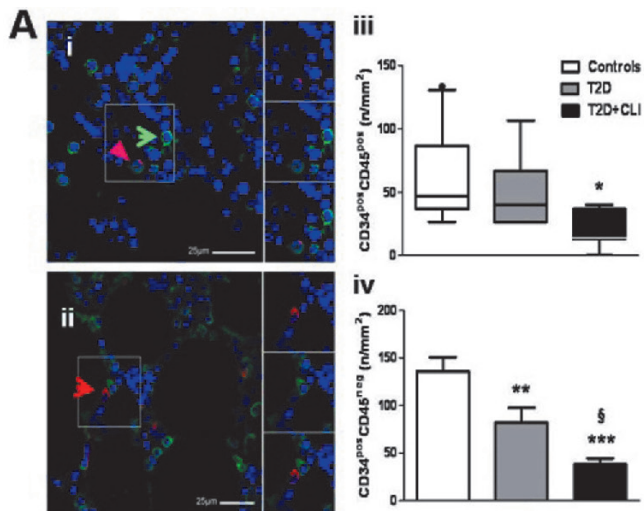
## 122

**Type 2 diabetes alters the marrow vascular niche and stem cell content through interference with mechanisms controlling cell cycle and survival**

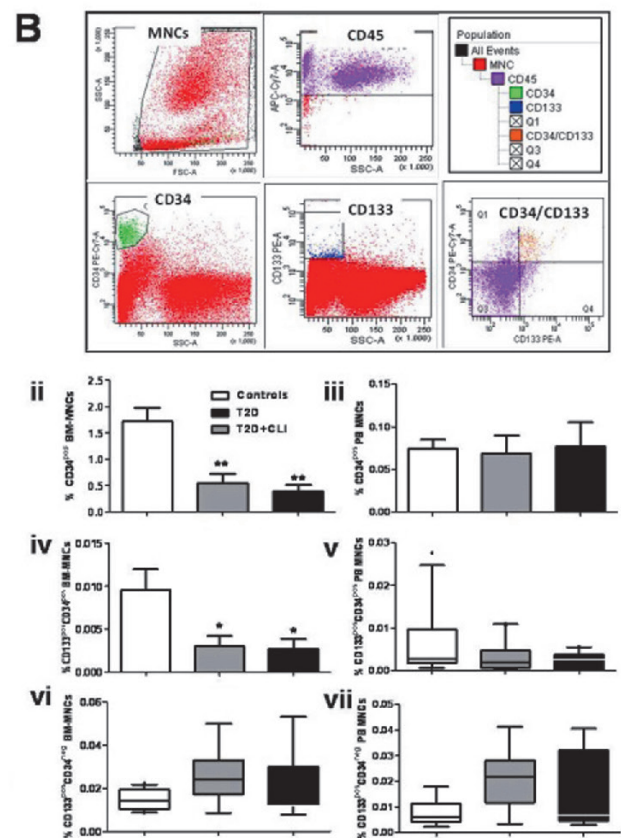
D. Cordella<sup>1</sup>, G. Spinetti<sup>1</sup>, O. Fortunato<sup>1</sup>, E. Sangalli<sup>1</sup>, S. Losa<sup>1</sup>, A. Gotti<sup>1</sup>, F. Carnelli<sup>1</sup>, F. Rosa<sup>1</sup>, A.P. Beltrami<sup>2</sup>, C. Emanueli<sup>3</sup>, P. Madeddu<sup>3</sup>;  
<sup>1</sup>IRCCS MultiMedica, Milan, Italy, <sup>2</sup>University of Udine, Italy, <sup>3</sup>University of Bristol, UK.

**Background and aims:** Circulating progenitor cells (PCs) are reduced in diabetic patients, but underlying mechanisms remain obscure. Here, we propose that diabetes directly affects bone marrow (BM) integrity by impinging upon the miR155/FOXO/p21/p27<sup>kip1</sup> signaling pathway.

**Materials and methods:** BM was obtained from age-matched non-diabetic controls (C) and type 2 diabetic (T2D) patients both undergoing hip replacement, and from T2D patients at occasion of amputation for critical limb ischemia (CLI). BM structure and cellular composition was assessed by histomorphometry, immunostaining, and flow cytometry analyses. Percentage of apoptotic BM-MNCs was measured on paraffin embedded tissue by TUNEL assay. Expression of miR-155 and miR-155 target gene FOXO3A as well as cyclin inhibitors p21 and p27<sup>kip1</sup> was determined in MAC-sorted CD34<sup>pos</sup> PCs. **Results:** Histomorphometry showed that the hematopoietic fraction is reduced in T2D (32%) and T2D+CLI (22%) as compared with C (52%) with fat replacement and bone rarefaction ( $p<0.01$ ). Vascular density was also reduced in both diabetic subgroups ( $p<0.05$ ) with the most prominent defect at capillary level (T2D:  $13\pm4$ , T2D+CLI:  $12\pm2$  and C:  $25\pm2$  cap/mm<sup>2</sup>). Immunofluorescence microscopy and flow cytometry documented the reduction of CD34<sup>pos</sup>CD45<sup>pos</sup> PCs in BM of both diabetic groups, whereas primitive CD133<sup>pos</sup>CD34<sup>neg</sup>CD45<sup>pos</sup> cells did not differ in diabetic and control subjects (Figure 1). Pro-angiogenic PCs, including CD45<sup>dim</sup>CD34<sup>pos</sup>KDR<sup>pos</sup> and CD34<sup>pos</sup>CD14<sup>pos</sup>CD45<sup>dim</sup>KDR<sup>pos</sup>CXCR4<sup>pos</sup> cells, were reduced in BM of T2D ( $0.014\pm0.01\%$ ) T2D+CLI ( $0.023\pm0.008\%$ ) as compared to C ( $0.054\pm0.01\%$ ),  $p<0.05$ , and a similar behavior was observed in peripheral blood (data not shown). Apoptosis is activated in diabetic BM-MNCs especially in patients with CLI (C:  $3.73\pm0.16$ ,  $n=6$ ; T2D:  $5.07\pm0.6$ ,  $n=5$ ; T2D+CLI:  $25.61\pm4.94$ ,  $n=5$ ; % of nuclei positive for DNA fragmentation;  $p<0.05$  vs. C). In line, CD34<sup>pos</sup> PCs sorted from BM of diabetic subjects showed upregulation of the pro-apoptotic factor FOXO3A (T2D: 51-folds and T2D+CLI: 118-folds vs. C,  $n=6$  per group) and downstream cyclin inhibitors, p21 (T2D: 118-folds and T2D+CLI: 79-folds vs. C,  $n=5$ ) and p27<sup>kip1</sup> (T2D: 29-folds and T2D+CLI: 19-folds vs. C,  $n=5$ ;  $p<0.05$  for all comparisons). Moreover, we found that miR-155 is downregulated in CD34<sup>pos</sup> PCs that show FOXO3A levels beyond the 95 percentile of normal distribution. **Conclusion:** Diabetes directly impinges upon BM integrity, causing microvascular rarefaction, shortage of pro-angiogenic cells and activation of pro-apoptotic signalling pathway. These data provide a new key of interpretation for failure of BM to provide an adequate source of regenerative cells in diabetes.



**Figure 1. Reduced abundance of CD34<sup>pos</sup> cells in BM of T2D patients.** A) Representative confocal microscopy photographs showing the presence of CD34<sup>pos</sup> cells among the CD45<sup>pos</sup> (i) and CD45<sup>neg</sup> (ii) cell fractions: CD45<sup>pos</sup> (green arrow), CD45<sup>pos</sup>CD45<sup>pos</sup> (pink arrowhead) and CD34<sup>pos</sup>CD45<sup>neg</sup> cells (red arrow). Nuclei are stained blue with DAPI. Bar graphs showing the abundance of CD34<sup>pos</sup>CD45<sup>pos</sup> cells (iii, median and 5–95% distribution) and CD34<sup>pos</sup>CD45<sup>neg</sup> cells (iv, mean $\pm$ SEM). B) Flow cytometry analysis of BM and PB cells: gating strategy of multicolor flow cytometry (i) and bar graphs showing the abundance of CD34<sup>pos</sup>CD45<sup>pos</sup> (ii&iii), CD34<sup>pos</sup>CD133<sup>pos</sup>CD45<sup>pos</sup> cells (iv&v) and CD34<sup>neg</sup>CD133<sup>pos</sup>CD45<sup>pos</sup> cells. \* $p<0.05$  and \*\* $p<0.01$  vs. controls. Controls,  $n=10$ ; T2D,  $n=7$ ; T2D+CLI,  $n=10$ .



## 123

### microRNA-15a and -16 are increased in circulating pro-angiogenic cells (PACs) of diabetic patients with critical limb ischaemia and impair PAC functions

G. Spinetti<sup>1</sup>, A. Caporali<sup>2</sup>, M. Meloni<sup>2</sup>, O. Fortunato<sup>1</sup>, M. Marchetti<sup>2</sup>, E. Sangalli<sup>1</sup>, E. Faglia<sup>1</sup>, P. Madeddu<sup>2</sup>, C. Emanuelli<sup>2</sup>

<sup>1</sup>IRCCS MultiMedica, Milan, Italy, <sup>2</sup>University of Bristol, UK.

**Background and aims:** Although effective endovascular and surgical therapies have been developed, major amputation is often needed for a large portion of diabetic patients with critical limb ischemia (CLI). Thus, cell therapy to restore blood flow is currently investigated. Relevant to this, number and function of circulating Pro-Angiogenic Cells (PACs) originated from bone marrow is impaired by the disease state with mechanisms that are still poorly understood. Recently, microRNAs (miRs) have been shown to regulate endothelial cell (EC) activities. Here, we aimed to obtain the first characterization human PAC miRs signature associated with CLI and type 2 diabetes (T2D). We further characterized functional impact of miR-15a and -16 on PAC and investigated miR-15a/16 target genes.

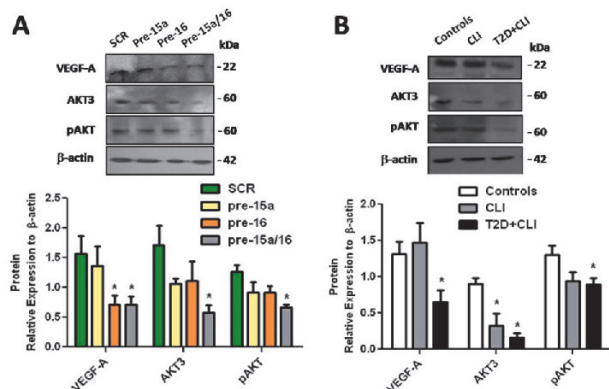
**Materials and methods:** PACs from healthy donors (C) and patients with CLI with/without T2D were enriched by culturing blood mononuclear cells on fibronectin in EC culture medium for 4 days. PACs were screened for 28 miRs expression by TaqMan q-RT-PCR ( $n=7$  samples per group). miR-15a and -16 were further measured in PACs from larger numbers of donors (C:  $n=17$ ; T2D+CLI;  $n=56$ , and non-diabetic CLI:  $n=16$ ). Next, miR-15a and 16 impact on PACs was studied by two complementary approaches: 1) C-PACs were transfected with pre-miR mimics or scramble control and 2) T2D+CLI-PACs with miR inhibitors or scramble. Transwell chambers assay was employed to study PAC migration toward SDF-1 (100 ng/mL), FBS (10%), VEGF-A (100ng/ml), bFGF (100ng/ml) or BSA (control). Bioinformatic analysis using MiRanda, Target Scan, Diana and miRBase softwares was used to predict miR-15a and -16 target genes.

**Results:** PACs from T2D-CLI patients showed de-regulation of several miRs. We focused on miR-15a and miR-16, which were both upregulated in diseased PACs, as also confirmed in the larger number of donors ( $p<0.05$ ). miR-15a and -16 expression in PACs of T2D and non-diabetic CLI ( $n=16$ ) patients were similar ( $p=NS$ ). Overexpression of both miR-15a/16 in healthy-PACs



decreased PACs migration ability toward FBS (57%), SDF-1 (75%), bFGF (59%), or VEGF-A (56%) ( $n=5$ ,  $p<0.05$  for all comparison vs scramble). In line, miR-15a/16 inhibition improved the migratory capacity of T2D+CLI-PACs ( $p<0.05$  vs scramble for all comparisons). VEGF-A and AKT3 were found to be predicted direct target of miR-15a and -16. Moreover, healthy PACs transfected with pre-miR15a/16 as well as patient-derived-PACs showed decreased protein expression of VEGF-A, AKT3, and phospho (p)-AKT (Figure 1).

**Conclusion:** miR-15a and miR-16 are upregulated in PACs of CLI patients (with/out T2D) and impair PAC pro-angiogenic potential, possibly by targeting VEGF-A and AKT-3. Thus miR-15a/-16 may represent new molecular target for *ex-vivo* manipulation of autologous cells used for vascular regeneration.



**Fig. 1. PACs expression of VEGF-A and AKT3, predicted targets of miR-15a and -16.** Western blot analyses of VEGF-A, AKT3 and phospho (p)-AKT in **A**) healthy PACs overexpressing miR-15a and -16 or transfected with SCR control ( $n=9$  subjects,  $*p<0.05$  vs. SCR), and **B**) in PACs from healthy, CLI and CLI+T2D donors ( $n=6$  subjects,  $*p<0.05$  vs. control). **Upper panels:** representative experiments, **lower panels:** bar graphs showing average data  $\pm$  SEM.

Clinical Trial Registration Number: NCT01269580

Supported by: 16928SAL-32

## 124

### Effects of n-3 polyunsaturated fatty acids on endothelial progenitor cell viability, function and inflammatory molecule expression

V. Spigoni<sup>1</sup>, A. Picconi<sup>2</sup>, M. Cito<sup>2</sup>, V. Ridolfi<sup>2</sup>, C. Casali<sup>2</sup>, E. Usberti<sup>2</sup>, I. Zavaroni<sup>2</sup>, L. Gnudi<sup>3</sup>, M. Metra<sup>1</sup>, A. Dei Cas<sup>2</sup>;

<sup>1</sup>Experimental and Applied Medicine, University of Brescia, Italy,

<sup>2</sup>Department of Internal Medicine and Biomedical Sciences, University of Parma, Italy, <sup>3</sup>Cardiovascular Division, King's College, London, UK.

**Background and aims:** Impairment in the number and function of endothelial progenitor cells (EPCs) has been shown in conditions characterized by an increased cardiovascular (CV) risk such as diabetes. N-3 polyunsaturated fatty acids (PUFA) retain beneficial CV effects due to their antioxidant and anti-inflammatory properties and to their ability to improve endothelial function. No studies are available investigating the effects of these agents on EPC biology. Aim of the study was to evaluate the effects of n-3 PUFA on early and late-outgrowth EPCs cultured *ex vivo*, in terms of viability, function and inflammatory response.

**Materials and methods:** EPCs were obtained from healthy donor buffy coats ( $n=14$ ). Early EPCs were obtained after culturing lymphomonocytes on fibronectin-coated dishes in EGM-2 for 7 days. In the same culture conditions late-outgrowth EPCs were obtained after 3 weeks. EPCs were phenotypically characterized by immunofluorescence and FACS analyses. AcLDL-uptake and lectin-binding capacity were tested. N-3 PUFA (10 $\mu$ M) (DHA:EPA = 1.5:0.9) or vehicle (BSA solution) were added to the culture media to test omega3 effects on EPC viability/proliferation, apoptosis, and function (tube-formation assay by co-plating cells on Matrigel with HUVEC). The effect of n-3 PUFA on pro-inflammatory molecule expression was also tested.

**Results:** The addition of PUFA improved early and late-outgrowth EPC proliferation by 38% ( $p=0.003$ ) and 128% ( $p=0.004$ ) respectively compared to vehicle with negligible effect apoptosis induction. Of note, PUFA prevented H<sub>2</sub>O<sub>2</sub>-induced cell death of late-outgrowth EPCs ( $p=0.004$ ). Furthermore n-3 PUFA enhanced early ( $p=0.015$ ) and late-outgrowth ( $p=0.029$ ) EPC capacity to form tubular-like structures. In the presence of n-3 PUFA, we observed a significantly lower expression of adhesion molecules in early (ICAM-1

$p<0.023$ ) and late-outgrowth (ICAM  $p<0.045$ ; VCAM  $p<0.025$ ) EPCs and of pro-inflammatory cytokines (MCP-1, TNF $\alpha$  and IL8) ( $p<0.05$ ) compared to vehicle.

**Conclusion:** Our data show for the first time a direct beneficial effect of n3-PUFA on EPC biology suggesting a protective role of omega3 on the vascular system possibly mediated by the reduction of inflammation and/or oxidative stress.

## 125

### Exendin-4 inhibits palmitate-induced apoptosis of human cardiac progenitor cells

A. Leonardini, L. Laviola, M.R. Orlando, M. Incalza, A. Pescechiera, A. Natalicchio, S. Perrini, F. Giorgino;  
Endocrinology & Metabolic Diseases, University of Bari, Italy.

**Background and aims:** Multipotent cardiac progenitor cells (CPCs) can be isolated from the adult heart, thus providing a constant tissue renewal. CPC dysfunction and increased apoptosis may contribute to functional abnormalities of the heart observed in metabolic diseases. There is growing evidence suggesting that glucagon-like peptide-1 (GLP-1) and GLP-1-based therapies may promote survival of cardiac cells in environmental stress conditions. The aim of this study was to investigate the protective effects of the GLP-1 receptor agonist exendin-4 on palmitate-induced apoptosis in human CPCs isolated from adult heart biopsies, obtained from the auricle in the course of open heart surgery.

**Materials and methods:** Biopsy-obtained cells showed typical features of mesenchymal multipotent cells, including the differentiation potential toward the adipogenic, osteogenic and chondrogenic lineages, and expression of specific surface markers by flow-cytometry and of lineage-specific intracellular markers GATA-4 and MEF-2C by quantitative real-time PCR. Expression and activation levels of the proteins under investigation were evaluated by immunoblotting techniques. Cell apoptosis was detected by measuring cytosolic oligosomes. Silencing of the GLP-1 receptor was obtained by using specific siRNAs.

**Results:** Acute exposure of CPCs to GLP-1 resulted in a time- and dose-dependent increase in intracellular cAMP levels and CREB phosphorylation, which were abolished in the presence of the GLP-1 receptor antagonist exendin 9-39 or in cells with siRNA-mediated silencing of the GLP-1 receptor. When CPCs were incubated in the presence of 25 mM palmitate for 16 h, a 4-fold increase in cell apoptosis was observed ( $p<0.05$ ). Under the same experimental conditions, incubation with palmitate for shorter times (10-120 min) did not change cell apoptosis. In addition, exposure to 25 mM glucose for up to 16 h had no effect on CPC apoptosis. Preincubation of CPCs with the GLP-1 receptor analogue exendin-4 (20 nM for 16 h) inhibited palmitate-induced apoptosis ( $p<0.05$  vs. palmitate-treated CPCs).

**Conclusion:** Excess palmitate reduces the viability of cardiac precursor cells, whereas high glucose has no effect. Activation of canonical GLP-1 receptors in human cardiac precursors exerts anti-apoptotic actions in the presence of lipotoxicity; this mechanism may contribute to the reported cardioprotective effects of GLP-1 receptor agonists.

Supported by: Lilly Foundation, Italy; Fo.Ri.SID

## 126

### Dipeptidyl peptidase-4 deficiency restores the impaired post-ischaemic mobilisation of haematopoietic and endothelial progenitors in experimental diabetes

M. Albiero<sup>1,2</sup>, L. Menegazzo<sup>1,2</sup>, C. Agostini<sup>1,2</sup>, A. Avogaro<sup>1,2</sup>, G.P. Fadini<sup>1,2</sup>;  
<sup>1</sup>Department of Medicine, University of Padova, <sup>2</sup>Venetian Institute of Molecular Medicine, Padova, Italy.

**Background and aims:** Vascular stem/progenitor cells contribute to cardiovascular homeostasis and are reduced in diabetic patients. Bone marrow mobilization of progenitor cells, which relies on the regulation of SDF-1 $\alpha$ , is impaired in diabetes. SDF-1 $\alpha$  is one substrate of dipeptidyl peptidase (DPP)-4 and diabetic patients have an increased DPP-4 activity. We aimed to determine whether DPP-4 deficiency is able to restore post-ischemic mobilization of progenitor cells in diabetic rats.

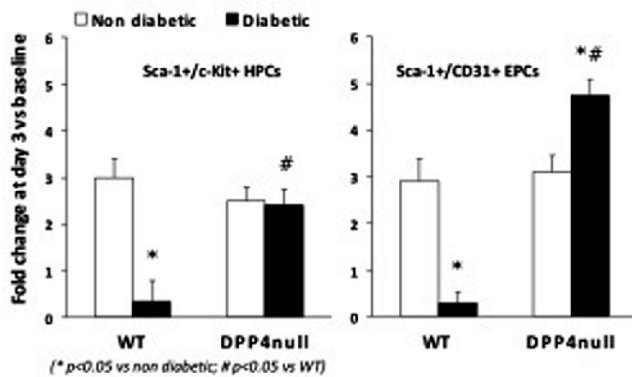
**Materials and methods:** We performed quantification of circulating hematopoietic (Sca-1+/c-Kit+) and endothelial (Sca-1+/CD31+) progenitor cells in diabetic and non-diabetic control (wt) and DPP-4 deficient (DPP4null) F344 rats, before and at 3 and 7 days after induction of hind limb ischemia. Dia-



betes was induced by intraperitoneal streptozotocin and experiments were performed 4 weeks after confirmation of hyperglycemia. Muscle capillary density was determined by CD31 immunostaining at day 7 after ischemia.

**Results:** Blood glucose and HbA1c were markedly elevated in diabetic versus non diabetic rats, but there were no differences in DPP4null versus wt animals. DPP-4 activity in DPP4null rats was about 10% of that in wt rats. In wt non diabetic animals, Sca-1+/c-Kit+ and Sca-1+/CD31+ progenitor cells significantly increased 3-fold at day 3 and returned at baseline levels 7 days after ischemia. In wt diabetic rats, progenitor cell mobilization was completely impaired and there were paradoxical reductions in Sca-1+/c-Kit+ and Sca-1+/CD31+ cells at day 3 compared to baseline. In DPP4null diabetic rats, progenitor cell mobilization was restored to levels higher than normal. Recovery of capillary density was impaired in wt diabetic versus non diabetic rats, while it was not significantly reduced in DPP4null diabetic versus non diabetic rats.

**Conclusion:** DPP-4 deficiency restores the impaired post-ischemic mobilization of hematopoietic and endothelial progenitor cells in streptozotocin diabetic rats. This is paralleled by a preserved recovery in the microvascular network of the ischemic tissue.



Supported by: EFSD/Lilly fellowship to GFP

## OP 22 Mitochondria and insulin action

127

### Transcriptional regulation of mitochondrial biogenesis in white adipose tissue and control of glucose homeostasis

J.A. Villena<sup>1,2</sup>, N. Enguix<sup>1,2</sup>, R. Pardo<sup>1</sup>, A. González<sup>1</sup>, R. Simó<sup>1,2</sup>, A. Kralli<sup>3</sup>,  
<sup>1</sup>Vall d'Hebron-Research Institute, Barcelona, Spain, <sup>2</sup>CIBERDEM, Barcelona, Spain, <sup>3</sup>The Scripps Research Institute, La Jolla, USA.

**Background and aims:** The endocrine function of adipocytes and their capacity to store triglycerides and prevent ectopic lipid deposition have been shown to be fundamental in the control of glucose homeostasis. In addition, compelling evidence suggests that the mitochondrial oxidative capacity of adipocytes may also exert a great influence on whole body insulin sensitivity. Indeed, mitochondrial dysfunction has been observed in white adipose tissue (WAT) of insulin-resistant individuals, as well as in distinct rodent models of type 2 diabetes. Consistent with an impairment of mitochondrial function, clinical studies have shown that patients with insulin resistance or type 2 diabetes have reduced mitochondrial mass in WAT. Mitochondrial biogenesis is primarily modulated at the transcriptional level by several transcription factors whose activity is coordinated by members of the PGC-1 family of co-activators. The reduced levels of PGC-1α found in WAT of insulin-resistant patients has led to suggest that decreased PGC-1α expression, and the consequent mitochondrial dysfunction, could contribute to the development of type 2 diabetes. However, using an adipose-specific PGC-1α knockout mouse model, we have recently shown that PGC-1α is not required to sustain basal mitochondrial gene expression or function in white adipocytes. We have also shown that PGC-1α is dispensable for rosiglitazone-induced mitochondrial biogenesis in WAT, suggesting that other transcriptional regulators may play a more significant role in the regulation of mitochondrial gene expression in this tissue. Here, we address the function of PGC-1β in WAT and its contribution to the regulation of whole body energy and glucose homeostasis.

**Materials and methods:** To assess *in vivo* the role of PGC-1β in WAT, we have generated a mouse model in which the *ppargc1b* gene has been ablated by homologous recombination specifically in adipocytes (PGC1β-FAT-KO mice). We have also used cultured 3T3-L1 adipocytes in which PGC-1 co-activators have been knockeddown by the use of specific siRNAs to study their contribution to the adipocyte oxidative capacity.

**Results:** Gene expression profiling revealed a significant enrichment in mitochondrial genes among the genes down-regulated in WAT of PGC1β-FAT-KO mice. Interestingly, the PGC-1β target genes identified were mostly restricted to few pathways, such as oxidative phosphorylation and the tricarboxylic acid cycle. Consistent with decreased gene expression, mitochondrial protein content and activity were decreased in WAT of PGC1β-FAT-KO. Moreover, contrary to what we have previously shown for PGC-1α, induction of mitochondrial genes by rosiglitazone was blunted in PGC1β-FAT-KO mice. Similarly, knockdown of PGC-1β in 3T3-L1 adipocytes reduced both, basal and rosiglitazone-induced mitochondrial gene expression and oxygen consumption. However, knockdown of PGC-1α had no effect. Despite the decreased mitochondrial function in WAT, PGC-1β-FAT-KO mice did not exhibit insulin resistance.

**Conclusion:** Our results show that, in WAT, PGC-1β is essential to sustain basal and rosiglitazone-induced expression of mitochondrial genes involved in ATP production. Although lack of PGC-1β impairs adipocyte oxidative capacity, is not sufficient to induce obesity or whole body insulin resistance.

Supported by: Grants SAF2008-03644 and RYC2006-0022622 from MICINN to J.A.V.

128

### Effects of the mitochondrial Atp8 mutation on metabolic parameters, life span and expression of respiratory chain complexes after long term high fat diet

H. Weiss, F. Nulle, S. Baltrusch, M. Tiedge;

Institute of Medical Biochemistry, University of Rostock, Germany.

**Background and aims:** The conplastic mouse strain B6-mt<sup>FVB</sup> carries a mitochondrial DNA mutation in the *Atp8* gene coding for the regulatory subunit 8 of ATP-synthase. In comparison to its control strain B6-mt<sup>AKR</sup> the B6-mt<sup>FVB</sup> strain shows impaired glucose tolerance

in response to metabolic stress, induced by high fat diet (HFD). It was the aim of this study to investigate the effects of a long term HFD on glucose/lipid metabolism, expression of respiratory chain complexes in liver and life span. **Materials and methods:** B6-mt<sup>AKR</sup> (AKR) and B6-mt<sup>FVB</sup> (FVB) mice were fed high fat (HFD, 60% fat) or control diet (CD, 10% fat) directly after weaning (week 5). Serum insulin, leptin, triglycerides, cholesterol, i.p. glucose tolerance, i.p. insulin sensitivity, and beta cell mass were investigated at 3, 6, and 12 month of HFD. Respiratory chain complex proteins were quantified by Western blot analyses. Data were analysed by t-test, ANOVA and log rank test.

**Results:** Serum insulin levels were 3-fold elevated in FVB ( $p<0.01$ ) animals already after 3 month of HFD in comparison to CD. Serum insulin levels in AKR mice showed this increase delayed after 6 month of HFD. After 9 and 12 month of HFD serum insulin levels were not different to the CD fed animals in the AKR strain while in FVB mice HFD resulted in a 9-fold increase in serum insulin levels compared to CD ( $p<0.05$ ). The increase of serum insulin levels in HFD fed AKR animals was accompanied by a 3-fold increase of beta cell mass at 6 month of age ( $p<0.001$ ). After 12 month of HFD FVB mice showed a 2-fold increase in serum insulin and 3-fold increase in beta cell mass compared to the situation after 6 month HFD. HFD resulted in significantly elevated serum cholesterol levels after 6 months (FVB:  $p<0.001$ ; AKR:  $p<0.01$ ) and 12 months (FVB/AKR:  $p<0.05$ ) in both strains, while serum triglyceride levels were not affected by HFD at any time point. HFD induced a significant increase of serum leptin levels in FVB mice ( $89 \pm 8$  ng/ml) compared to AKR mice ( $65 \pm 5$  ng/ml;  $p<0.05$ ) animals. After 12 months of HFD serum leptin levels normalized in the AKR strain but remained high in FVB mice. Interestingly, i.p. glucose tolerance and i.p. insulin sensitivity were impaired after 3 months of HFD but normalized gradually until 12 months of HFD in both strains. However, the survival time of HFD fed FVB mice was significantly reduced (68 weeks vs. 80 weeks,  $p<0.03$ ) compared to AKR control mice. Expression levels of respiratory chain complexes in liver showed a significant increase in FVB animals between 3 and 6 months for complex I ( $p<0.01$ ) and complex V ( $p<0.05$ ), but normalized thereafter to the level of AKR controls.

**Conclusion:** The data indicate that the Atp 8 mutation in the ATP-synthase induces a high sensitivity to metabolic stress in early lifetime. In this period compensatory increases of OXPHOS complexes confer adaptation of a pathologic mitochondrial phenotype to metabolic challenges. Interestingly, metabolic parameters normalized despite ongoing HFD. After 12 months of HFD decompensation occurs rapidly with a decreased survival rate of FVB mice. Thus, metabolic challenges at early lifetime could induce changes in mitochondrial dynamics that ultimately will reduce life. Thus, compensated mitochondrial dysfunction in obese adolescents can initiate programs that predispose to mitochondrially-based organ failures at old age.

Supported by: BMBF, DDG

## 129

### Increased lipolysis is associated with enhanced hepatic mitochondrial function and insulin resistance in a mouse model of type 1 diabetes

G.F.B. Séquaris<sup>1</sup>, T. Jelenik<sup>1</sup>, E. Phielix<sup>1</sup>, J. Kotzka<sup>2</sup>, B. Knebel<sup>2</sup>, M. Ouwens<sup>2</sup>, J. Wei<sup>3</sup>, A.L. Reinbeck<sup>1</sup>, P. Nowotny<sup>1</sup>, H.-J. Partke<sup>1</sup>, M. Roden<sup>1,3</sup>, J. Szendroedi<sup>1,3</sup>

<sup>1</sup>Institute for Clinical Diabetology, German Diabetes Center, <sup>2</sup>Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, <sup>3</sup>Department of Metabolic Diseases, University Clinics Düsseldorf, Düsseldorf, Germany.

**Background and aims:** Long-standing type 1 diabetes (T1D) frequently associates with insulin resistance. Although glucose toxicity is held responsible, the mechanism underlying insulin resistance in T1D are not understood. Thus, we aimed to assess the relative roles of glucose and lipid metabolism and mitochondrial function in non-obese diabetic mice (NOD) at three days after the onset of diabetes (short-term diabetic NOD,  $n=20$ ) or in the normoglycaemic state (non-diabetic NOD,  $n=20$ ) and in wild-type C57BL/6 mice (CON,  $n=23$ ) under fasted and postprandial conditions.

**Material and methods:** Muscle and liver insulin sensitivity was measured with hyperinsulinaemic-euglycaemic clamps combined with deuterated glucose. Mitochondrial function was assessed by high-resolution respirometry, mitochondrial biogenesis from peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$  (PGC-1 $\alpha$ ), transcription factor A, mitochondrial (TFAM) and nuclear respiratory factor 1 (NRF1) by quantitative RT-PCR and mitochondrial content from the ratio of mitochondrial to nuclear DNA using quantitative RT-PCR. Cellular signaling pathways were analysed from phosphorylated c-Jun N-terminal kinase (phospho-JNK), suppressor of cy-

tokine signaling 3 (SOCS3) and insulin receptor substrate-1 (IRS-1) using immunoblotting.

**Results:** Short-term diabetic NOD had lower muscle (-56%,  $p<0.05$  vs. CON and -44%,  $p<0.05$  vs. non-diabetic NOD), but not hepatic insulin sensitivity. They also displayed increased fasting serum triglycerides (+52%,  $p<0.01$  vs. CON and +66%,  $p<0.01$  vs. non-diabetic NOD) and free fatty acids (+57%,  $p<0.01$  vs. CON and +63%,  $p<0.05$  vs. non-diabetic NOD). Furthermore, visceral and subcutaneous fat tissue weights were 63% ( $p<0.01$ ) and 75% ( $p<0.01$ ) lower in diabetic NOD than in CON. Diabetic NOD had 2.4fold and 1.6fold greater hepatic oxidative capacity than in CON ( $p<0.001$ ) and non-diabetic NOD ( $p<0.01$ ) under postprandial conditions. Hepatic mitochondrial biogenesis was also higher (PGC-1 $\alpha$ : 44fold, NRF1: 2.2fold and TFAM: 6.5fold,  $p<0.001$  vs. CON as well as 32fold, 6fold and 4.2fold,  $p<0.001$  vs. non-diabetic NOD). Muscle oxidative capacity was similar in all groups and mitochondrial content was not affected in both tissues. Phospho-JNK was increased by 156% and 72% ( $p<0.05$ ) in muscle and liver of short-term diabetic and non-diabetic NOD compared to CON mice. Likewise, SOCS3 protein levels were increased by 25% ( $p<0.05$  vs. CON) in skeletal muscle and by 20% ( $p<0.05$  vs. CON) in liver of short-term diabetic NOD mice. However, decreased expression of IRS-1 (-47%,  $p<0.01$  vs. CON) was noted only in muscle of diabetic NOD mice.

**Conclusion:** Insulin deficiency at the onset of diabetes causes lipolysis, thereby increasing systemic lipid availability, which stimulates hepatic mitochondrial oxidation. Subsequent activation of inflammatory signaling induces muscle insulin resistance in NOD mice.

Supported by: DZD e.V., DFG, Schmutzler Stiftung, Skróder Stiftung, EFSD/Lilly grant

## 130

### The “healthy” phenotype of mice with mitochondrial uncoupling in skeletal muscle is linked to increased substrate metabolism and induction of the antioxidant defence system

S. Keipert<sup>1</sup>, A. Chadt<sup>2</sup>, V. Ayala<sup>3</sup>, A. Voigt<sup>1</sup>, M. Ost<sup>1</sup>, M. Portero-Otín<sup>3</sup>, R. Pamplona<sup>3</sup>, H. Al-Hasani<sup>2</sup>, S. Klaus<sup>1</sup>

<sup>1</sup>German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany, <sup>2</sup>German Diabetes-Center, Düsseldorf, Germany, <sup>3</sup>Facultat de Medicina, Lleida, Spain.

**Background and aims:** Transgenic mice with ectopic expression of uncoupling protein 1 (UCP1) in skeletal muscle (SM) mitochondria showed on a high fat diet a delayed development of obesity, improved glucose tolerance and a dramatically 42% increased median lifespan in comparison to their wildtype (WT) littermates. The reason for the increased longevity is not clear so far. It is postulated that mild uncoupling through UCPs affects cellular mitochondrial ROS production and might thus impact aging. We could show a decreased ROS production in isolated mitochondria in UCP1 TG (TG) mice. Another hallmark of TG mice is a body weight independent increased insulin sensitivity. Insulin resistance plays a crucial part in the pathogenesis of the metabolic syndrome and is characterized by a reduced substrate metabolism and impaired defense against stress in skeletal muscle. The aim of this study was to clarify the molecular mechanisms in skeletal muscle which are responsible for the “healthy” phenotype of young UCP1 TG mice.

**Materials and methods:** We investigated body composition and food intake under a low fat (LF) and a high fat (HF) diet. At 20 wks of age (8 wks of dietary intervention) WT and TG mice were killed for SM analysis of substrate metabolism (AMP-activated protein kinase [AMPK], acetyl-CoA carboxylase [ACC], fatty acid translocase CD36, ex-vivo fatty acid oxidation), and oxidative stress parameters including antioxidant defense system (catalase and superoxide dismutase (SOD) activity, heat shock protein (HSP) expression) and oxidative damage markers (glutamic semialdehyde, amino adipic semialdehyde, Nε-[carboxymethyl] lysine, Nε-[carboxyethyl] lysine and Nε-[malondialdehyde] lysine measured by GC/MS).

**Results:** TG mice were resistant against high fat diet induced obesity in younger age. In contrast to WT, TG mice showed no increased body weight or body fat content but increased food intake on a high fat diet in comparison to a low fat diet. In TG animals AMPK activity in SM was significantly increased compared to WT. Furthermore, gene and protein expression analyses of SM pointed to a higher lipid metabolism which was confirmed by an increased ex-vivo fatty acid oxidation in two different muscle types (extensor digitorum longus +85%,  $p=0.004$ ; soleus +54%,  $p=0.038$  in TG mice compared to WT). Interestingly, in contrast to the decreased ROS production in isolated mitochondria, TG mice showed no decreased, but rather a slightly increased ROS damage especially concerning lipid peroxidation products in SM. This was

paralleled by an induction of the endogenous oxidative damage protection system as evident by an increased catalase (LF and HF diet) and SOD (only on HF diet) activity and increased HSP25 protein expression in SM.

**Conclusion:** Our data suggest that SM mitochondrial uncoupling leads to a “healthy” lean phenotype on a high fat diet by preserving insulin sensitivity through increased substrate metabolism and an induction of ROS-signaling pathways. This argues for the mitochondrial hormesis hypothesis suggesting that ROS are essential signaling molecules for health and longevity. In TG mice SM uncoupling seems to act as an exercise mimic leading to an increased energy supply and a mild stress induction which in turn increases the endogenous antioxidant defense system

Supported by: German Research Council (DFG) and the BIOCLAIMS Project (EU)

## 131

### Altered electron chain enzyme kinetics between lean, obese and type 2 diabetes mellitus, but no impaired mitochondrial function upon short-term lipid exposure in young humans

E. Phielix<sup>1</sup>, M. Zeppetzauer<sup>1</sup>, G. Sequaris<sup>1</sup>, T. Jelenik<sup>1</sup>, P. Nowotny<sup>1</sup>, J. Szendroedi<sup>1,2</sup>, M. Roden<sup>1,2</sup>

<sup>1</sup>Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf,

<sup>2</sup>Department of Metabolic Diseases, University Clinics Düsseldorf, Germany.

**Background and aims:** Patients with type 2 diabetes (T2D) generally have lower mitochondrial oxidative capacity and plasticity and insulin sensitivity than lean humans (CON). As T2D and lean humans infused with lipids feature increased circulating free fatty acids (FFA) and insulin resistance, lipotoxicity has been suggested to underlie lower mitochondrial function and insulin sensitivity. We examined muscle mitochondrial function in young, glucose tolerant CON, obese humans (OBE) and T2D and in CON upon short-term elevation of circulating FFA.

**Material and methods:** We included 29 CON, 9 OBE and 8 T2D humans (age: 29±2 years, BMI: 23.4±0.6 kg/m<sup>2</sup>, 29.3±2.2 years, 41.4±1.9 kg/m<sup>2</sup>, and 58.5±2.8 years, 35.6±1.2 kg/m<sup>2</sup>,  $p<0.05$  CON vs. OBE and T2D). Participants underwent clamps combined with deuterated glucose. Biopsies were taken from *vastus lateralis* at baseline and in a subgroup of 13 CON- upon 4 hours of Intralipid (20%) before the clamp. Mitochondrial function, including ADP-coupled oxidative capacity on glutamate and succinate (GS3) followed by FCCP-induced uncoupling (state u) as well as enzyme kinetics ( $K_m$ : substrate concentration at which the reaction rate is 50% of maximum speed and  $V_{max}$ : maximal velocity at which the enzyme catalyzes the reaction) of NADH and FADH<sub>2</sub> dehydrogenase, was examined in muscle fibers using high-resolution respirometry.

**Results:** CON were less insulin resistant (IR) than both T2D and OBE (M-value: 9.7±1.0 vs. 2.2±0.8 and 1.6±0.2 mg/kg/min,  $p<0.05$ ). G3 and GS3 were lower in T2D compared to CON (O<sub>2</sub>-flux: 29±4 and 62±8 pmol/mg/s, respectively,  $p<0.05$ ). OBE had a tendency towards lower G3 than CON (40±5 vs. 48±2 pmol/mg/s,  $p=0.06$ ), but no difference in GS3 (69.4±8.3 vs. 79.3±4.3 pmol/mg/s,  $p=ns$ ). State u was lower in T2D and OBE compared to CON (84±8, 97±5 vs. 113±5 pmol/mg/s,  $p<0.05$ ).  $V_{max}$  for NADH dehydrogenase was lower in T2D than in CON and OBE (24.7±9.0 vs. 56±3 and 44±6 pmol/mg/s,  $p<0.05$ ); the  $K_m$  was higher in OBE than in CON and T2D (4.5±0.9 vs. 5.5±0.4 and 2.2±0.5 μmol/l,  $p<0.05$ ). While  $V_{max}$  for FADH<sub>2</sub> dehydrogenase was not different between all groups (66±3, 66±3 and 67±9 pmol/mg/s),  $K_m$  was higher in both OBE and T2D compared to CON (7.6±0.6 and 8.1±2.3 vs. 5.5±0.4 μmol/l,  $p<0.05$ ). Elevation of plasma FFA from 0.48±0.05 to 2.12±0.19 mmol/l ( $p<0.01$ ) resulted in ~60% reduced insulin-stimulated glucose uptake (3.7±0.6 mlkg<sup>-1</sup>min<sup>-1</sup>) but did not affect state 3 and state u respiration (67±4 vs 62±5 pmolmg<sup>-1</sup>s<sup>-1</sup>; state u: 103±7 vs 100±11 pmolmg<sup>-1</sup>s<sup>-1</sup>,  $p=ns$  vs. baseline). Also, enzyme kinetics of NADH and FADH<sub>2</sub> dehydrogenases were not altered.

**Conclusions:** Increased substrate sensitivity of complex (C) 1 and C2 in obese IR but glucose tolerant humans might reflect adaptive upregulation of mitochondrial enzyme activity but reduced maximal capacity as already present in these patients. In patients with overt T2D, substrate sensitivity is increased in C2 but oxidative capacity is impaired in C1, C2 and uncoupled respiration. Although plasma FFA correlate with IR and short-term increase in circulating FFA cause IR, elevation of FFA does not affect mitochondrial function. This supports the contention that muscular IR does not require abnormal mitochondrial function per se.

Clinical Trial Registration Number: NCT01229059

Supported by: EFSD/Lilly grant

## 132

### Insulin sensitivity does not correlate with mitochondrial function: studies in gastric bypass patients

M.T. Lund<sup>1,2</sup>, M. Hansen<sup>1</sup>, R. Kraunsøe<sup>1</sup>, A. Floyd<sup>2</sup>, K. Bech<sup>2</sup>, F. Dela<sup>1</sup>

<sup>1</sup>Biomedical Sciences, University of Copenhagen, <sup>2</sup>Department of Surgery, Koege Hospital, Denmark.

**Background and aims:** Obesity and type 2 diabetes is accompanied by intramyocellular lipid accumulation. It has been hypothesized that this accumulation leads to mitochondrial dysfunction and insulin resistance. Whether mitochondrial dysfunction is actually present and a cause of insulin resistance is still being debated. Our aim was to compare changes in peripheral insulin sensitivity and mitochondrial respiration after a diet induced weight loss and subsequently by a Roux-En-Y gastric bypass induced weight loss in obese patients with or without type 2 diabetes.

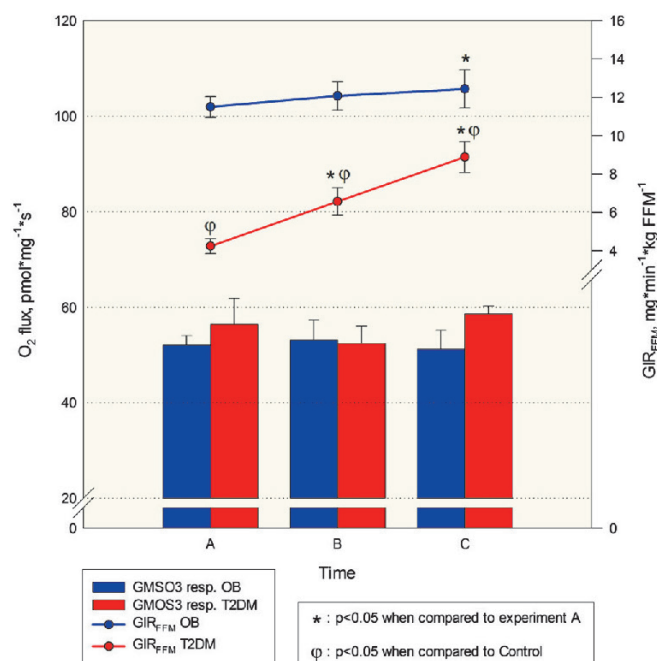
**Materials and methods:** 16 obese patients (4M/12F; 6 with (T2DM) and 10 (OB) without type 2 diabetes) reported thrice to the lab after an overnight fast: Prior to weight loss (A), 2 mo later just prior to operation (B) and 4 mo after operation (C). At each visit three tests were performed: Day 1: Dual Energy X-ray Absorption Scan for body composition and graded bicycle test on a stationary bike for VO<sub>2max</sub>. Day 2: Hyperinsulinaemic euglycemic clamp to measure peripheral insulin sensitivity. Test days were separated by at least 48h. A biopsy were taken from m. vastus lateralis for respirometry prior to the clamp. Muscle fibers were split, permeabilised and rinsed before transferred to the respirometer. Malate (2 mM) and glutamate (10 mM); ADP (5 mM) and magnesium (20 mM); octanoylcarnitine (1.5 mM); and succinate (10 mM) were added consecutively to measure maximally coupled respiration. This was follow by titration of FCCP (steps of 0.25 μM) for maximally uncoupled respiration. Two way ANOVA for repeated measures was used in comparison of the two groups over time.

**Results:** There were no baseline differences in age: 38±10 vs. 40±7 yrs; BMI: 43±1 vs. 41±2 Kg·m<sup>-2</sup>; Fat Free mass (FFM): 60±2 vs. 64±4 kg or VO<sub>2max</sub>: 2.7±0.2 vs. 2.7±0.3 L·min in OB and T2DM, respectively. Weight loss relative to baseline (A) was similar in both groups: 6±1 (1±0.3 kg FFM) vs. 5±1 (1±0.5 kg FFM) kg at exp B and 27±1 (5±0.7 kg FFM) vs. 28±4 (5±2 kg FFM) kg at exp C in OB and T2DM, respectively. VO<sub>2max</sub> did not change significantly during the experiment. Insulin sensitivity measured per fat free mass (GIR<sub>FFM</sub>) and maximally coupled respiration data in the two groups are shown in figure 1. Uncoupled respiration was similar at baseline: 66±3 vs. 67±5 pmol\* mg<sup>-1</sup>s<sup>-1</sup> in OB and T2DM, respectively, and did not change significantly during the experiment.

**Conclusion:** In spite of the marked difference in insulin sensitivity between OB and T2DM, maximally coupled and uncoupled mitochondrial respiration was similar in the two groups. Moreover, with marked improvements in T2DM insulin sensitivity, due to the massive weight loss, mitochondrial respiration remained unchanged. The unchanged VO<sub>2max</sub> indicates that the patients do not take up exercise habits along with their weight loss. In conclusion, our results speak against an association of mitochondrial respiratory capacity and insulin resistance in skeletal muscle in obese and type 2 diabetic patients.



Figure 1. Mitochondrial respiration and Insulin sensitivity



Supported by: The Danish Council for Strategic Research

## OP 23 Complications and biomarkers

### 133

#### Plasma advanced glycation end products are not associated with cardiovascular disease in individuals with or without type 2 diabetes: the Hoorn and CODAM studies

N. Hanssen<sup>1</sup>, L. Engelen<sup>1</sup>, I. Ferreira<sup>1,2</sup>, J. Scheijen<sup>1</sup>, M. Huijberts<sup>1</sup>, M. van Greevenbroek<sup>1</sup>, C. van der Kallen<sup>1</sup>, J. Dekker<sup>3</sup>, G. Nijpels<sup>3</sup>, C. Stehouwer<sup>1</sup>, C. Schalkwijk<sup>1</sup>;

<sup>1</sup>Internal medicine, Maastricht University, <sup>2</sup>Clinical Epidemiology and Medical Technology Assessment, Maastricht University, <sup>3</sup>Epidemiology and Biostatistics, VU University Medical Centre, Amsterdam, Netherlands.

**Background and aims:** Advanced glycation endproducts (AGEs) may contribute to cardiovascular disease (CVD), particularly among individuals with type 2 diabetes (DM2). However, epidemiological evidence supporting an adverse association between increased levels of AGEs and CVD or subclinical markers thereof, in individuals with or without DM2, remains unclear. We therefore investigated, in a large sample of participants with various degrees of glucose metabolism, the associations of plasma concentrations of protein-bound and free forms of AGEs with prevalent CVD and carotid intima-media thickness (IMT) and ankle-brachial index (ABI).

**Materials and methods:** We measured plasma levels of the protein-bound AGEs N<sup>ε</sup>-(carboxymethyl)lysine (CML), N<sup>ε</sup>-(carboxyethyl)lysine (CEL) and free CML, CEL and 5-hydroxy-5-methylimidazole (MG-H1) with ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) and protein-bound pentosidine with high-performance liquid chromatography (HPLC), in participants from the Cohort Diabetes and Atherosclerosis Maastricht (CODAM) and the Hoorn Studies. The total combined study sample consisted of 1,291 individuals (mean age 64.5 ± 8.6 years, 45% women) of whom 44% had normal glucose metabolism (NGM), 23% had impaired glucose metabolism (IGM) and 33% had DM2. Data were analysed with multiple linear regression analyses, including adjustments for age, sex, glucose metabolism status and cohort and further for smoking, central obesity, blood pressure, blood lipids, renal function, smoking and use of medication.

**Results:** Protein-bound pentosidine [0.48 (IQR: 0.40–0.59) vs. 0.53 (0.43–0.67) nmol/mmol lysine,  $p=0.013$ ], and free CEL [45 (36–56) vs. 48 (39–62) nmol/l,  $p=0.007$ ] and MG-H1 [116 (84–165) vs. 141 (96–209) nmol/l,  $p<0.001$ ] were significantly increased in individuals with CVD (43% of the study population), but these differences disappeared after adjustment for cohort, age, sex, glucose metabolism status and other traditional cardiovascular risk factors. Plasma protein-bound AGEs (i.e. CML, CEL and pentosidine) and free CEL and MG-H1 were also not independently associated with carotid IMT or ABI, except for a positive association between the free form of plasma CML and ABI [standardized  $\beta=0.106$  (95% CI: 0.064 to 0.148),  $p=0.011$ ]. We did not find any independent associations between the AGEs and study outcomes in analyses stratified according to individuals' glucose metabolism status.

**Conclusion:** Although experimental studies have shown the importance of AGEs in the development of diabetes-related CVD, we found no independent adverse associations of plasma AGEs with CVD, carotid IMT or ABI in two large population-based cohort studies of individuals with NGM, IGM and DM2.

Supported by: CTMM, PREDICcT, DHS, DDRF, DKF

### 134

#### Serum angiogenin which influences vascular repair is related to macrovascular complications of diabetes, but not to diabetes per se

F. Obendorf<sup>1</sup>, M. Ursli<sup>1</sup>, C. Hoebaus<sup>1</sup>, R. Kaltenboeck<sup>1</sup>, G. Pesau<sup>1</sup>, J.-M. Brix<sup>2</sup>, R. Koppensteiner<sup>1</sup>, G. Schernthaner<sup>2</sup>, G.-H. Schernthaner<sup>1</sup>;

<sup>1</sup>Angiology, Medical University of Vienna, <sup>2</sup>Rudolf Foundation Hospital, Vienna, Austria.

**Background and aims:** Patients with diabetes have impaired vascular regenerative function leading to different macrovascular complications like myocardial infarction, stroke or peripheral arterial disease. Angiogenin (ANG), a small polypeptide and potent inducer of vascular growth, is associated with type 2 diabetes and poor glycemic control. Previously, we showed a possible influence of ANG on different microvascular diabetes complications. The aim

of this study was to investigate angiogenin in peripheral arterial disease (PAD). **Materials and methods:** We measured serum levels of ANG in 160 participants, using a commercial available ELISA (R&D systems, Minneapolis, MN): 43 type 2 diabetes without PAD (DM, 44% female, age  $62 \pm 10$  years, HbA1c  $8.3 \pm 1.9$  rel.%, BMI  $30 \pm 5$ ), 35 type 2 diabetes with PAD (DM-PAD, 14% female, age  $69 \pm 8$ , HbA1c  $6.8 \pm 1.1$  rel.%, BMI  $29 \pm 5$ ), 43 PAD patients without DM (PAD, 34% female, age  $68 \pm 11$  years, HbA1c  $5.8 \pm 0.3$  rel.%, BMI  $26 \pm 4$ ) and 38 age-matched healthy controls (CO, 57% female, age  $62 \pm 11$ , HbA1c  $5.8 \pm 0.4$ , BMI  $28 \pm 5$ ). DM-PAD and PAD consisted of patients with Fontaine stage I and II. Data are presented as mean  $\pm$  SD. Statistical analyses included Kolmogorov-Smirnov test, Student's unpaired t-test, ANOVA, univariate correlation analysis, multivariate linear regression modeling and logistic regression modeling, as appropriate.

**Results:** PAD patients with ( $466 \pm 124$  ng/ml) and without DM ( $491 \pm 125$  ng/ml) had significant higher levels of ANG compared to healthy controls ( $367 \pm 144$  ng/ml,  $p < 0.05$ ) and patients suffering from diabetes without PAD ( $368 \pm 145$  ng/ml,  $p < 0.05$ ). ANG levels did not differ between DM and CO ( $p = 0.986$ ), as well as between DM-PAD and PAD ( $p = 0.372$ ). Overall, patients with PAD had significant higher levels of ANG compared to patients and healthy controls without PAD ( $480 \pm 125$  vs.  $369 \pm 143$  ng/ml,  $p < 0.001$ ). Although patients with PAD had significant higher levels of ANG, we failed to show a significant difference between Fontaine stage I and II patients ( $423 \pm 103$  vs.  $491 \pm 131$  ng/ml,  $p = 0.102$ ). Correlation analysis of PAD staging and all available quantitative variables revealed ANG ( $R = 0.392$ ,  $p < 0.001$ ), Age ( $R = 0.291$ ,  $p < 0.001$ ), HbA1c ( $R = -0.419$ ,  $p < 0.001$ ), cholesterol ( $R = -0.194$ ,  $p = 0.020$ ), LDL cholesterol ( $R = -0.200$ ,  $p = 0.016$ ), diastolic blood pressure ( $R = -0.216$ ,  $p = 0.020$ ), as well as mean ankle-brachial index ( $R = -0.540$ ,  $p < 0.001$ ) as significant associates. A logistic regression model for the development of PAD in patients with and without diabetes demonstrated ANG (odds ratio (OR) = 1.007,  $p = 0.001$ ), HbA1c (OR = 0.521,  $p = 0.001$ ) and BMI (OR = 0.861,  $p = 0.012$ ) as significant predictors. To assess and compare the power of prediction of PAD, OR per 1 STD change was calculated. Thus a 1 STD change in ANG, resulted in a 247% increased likelihood of development of PAD. In other words, per 100 ng/ml increase of serum ANG, the risk for PAD increased by 170%.

**Conclusion:** In our study cohort angiogenin was associated with the development and presence of macrovascular disease in patients with and without diabetes. Together with our previous findings, we hypothesize a possible influence of angiogenin on vascular repair function in microvascular and macrovascular diabetes complications.

## 135

### Copeptin as biomarker for cardiovascular and all-cause mortality in type 2 diabetes (ZODIAC-31)

I.J. Riphagen<sup>1</sup>, W.E. Boertien<sup>1</sup>, A. Alkhalaf<sup>2</sup>, N. Kleefstra<sup>1,2</sup>, R.T. Gansevoort<sup>1</sup>, K.H. Groenier<sup>2,3</sup>, K.J.J. van Hateren<sup>2</sup>, J. Struck<sup>4</sup>, G. Navis<sup>1</sup>, H.J.G. Bilo<sup>1,2</sup>, S.J.L. Bakker<sup>1</sup>

<sup>1</sup>Internal Medicine, UMCG, Groningen, Netherlands, <sup>2</sup>Diabetes Center, Isala Clinics, Zwolle, Netherlands, <sup>3</sup>General Practice, UMCG, Groningen, Netherlands, <sup>4</sup>BRAHMS GmbH (Thermo Fisher Scientific), Hennigsdorf, Germany.

**Background and aims:** In acute heart failure and myocardial infarction, copeptin, a proximate marker for the antidiuretic stress-hormone vasopressin, has been reported to be of diagnostic and prognostic value. It is not known whether copeptin is also associated with outcome in stable outpatients. This is important, because a positive association would provide rationale for studies on reduction of cardiovascular risk by vasopressin antagonists that recently became available for chronic treatment. Our aim was to prospectively investigate whether copeptin is associated with cardiovascular (CV) and all-cause mortality when assessed from samples obtained during routine check-up of patients with diabetes mellitus type 2 (DM2).

**Materials and methods:** Patients with DM2 participating in the Zwolle Outpatient Project Integrating Available Care (ZODIAC) study were included. Cox regression analyses were used to investigate the association of baseline copeptin levels with cardiovascular and all-cause mortality.

**Results:** We included 1,265 patients (44% male, age  $67 \pm 11.6$  years). Copeptin concentrations were significantly higher in men than in women (median [IQR];  $7.4$  [ $4.4$ – $11.5$ ] versus  $4.1$  [ $2.7$ – $7.3$ ] pmol/L,  $p < 0.001$ ). After median follow-up for  $6.4$  [IQR  $3.2$ – $10.1$ ] years, 365 patients died (29%), with 154 deaths (12%) attributable to CV causes. A Kaplan-Meier analysis for gender-stratified tertiles of copeptin is shown in figure 1. In univariate cox regression analyses, log-transformed copeptin was significantly associated with

CV (Hazard Ratio (HR)(95%CI)=3.03 (1.99–4.62),  $p < 0.001$ ) and all-cause mortality (HR=1.55 (1.41–1.72),  $p < 0.001$ ). These associations remained significant after adjustment for CV risk factors including age, sex, BMI, smoking, systolic blood pressure, total cholesterol-HDL ratio, duration of diabetes, HbA<sub>1c</sub>, use of RAAS-inhibitors, history of CV disease, serum creatinine and urinary albumin-to-creatinine ratio, both for CV (HR=1.18 (1.00–1.39),  $p < 0.05$ ) and all-cause mortality (HR=1.22 (1.10–1.35),  $p < 0.001$ ).

**Conclusion:** We found copeptin to be independently associated with CV and all-cause mortality in patients with type 2 diabetes. Intervention studies should show whether the high CV risk in association with type 2 diabetes can be reduced by chronic treatment with vasopressin antagonists.

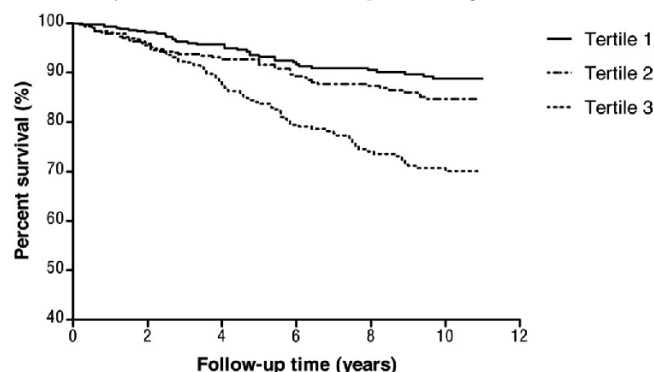


Figure 1. Kaplan-Meier survival curve for cardiovascular mortality according to gender-stratified tertiles of baseline copeptin levels.

Supported by: Center for Translational Molecular Medicine (CTMM), project PREDICCT

## 136

### Resistin, major cardiovascular events and all-cause mortality in patients with type 2 diabetes

C. Menzaghi<sup>1</sup>, S. Bacci<sup>2</sup>, L. Salvemini<sup>1</sup>, C. Powers<sup>3</sup>, A. Fontana<sup>4</sup>, S. De Cosmo<sup>2</sup>, F. Pellegrini<sup>4</sup>, A. Doria<sup>3,5</sup>, V. Trischitta<sup>1,6</sup>

<sup>1</sup>Research Unit of Diabetology and Endocrinology, IRCCS, San Giovanni Rotondo, Italy, <sup>2</sup>Unit of Endocrinology, IRCCS, San Giovanni Rotondo, Italy, <sup>3</sup>Joslin Diabetes Center, Boston, USA, <sup>4</sup>Unit of Biostatistics, IRCCS, San Giovanni Rotondo, Italy, <sup>5</sup>Harvard Medical School, Boston, USA, <sup>6</sup>"Sapienza" University, Rome, Italy.

**Background and aims:** We have recently shown that serum resistin is a strong and independent risk factor for incident major cardiovascular events (i.e. cardiovascular death, non fatal myocardial infarction-MI and non fatal stroke) in patients with type 2 diabetes (T2D) comprised in the Gargano Heart Study (GHS). As a first aim, we then decided to investigate whether, in this cohort, resistin improves the ability to predict such incident events as given by traditional clinical variables. In addition, given that cardiovascular disease (CVD) represents the first cause of death in diabetic patients, a second aim was to test the role of serum resistin as a risk factor for all-cause mortality in an independent cohort of patients with T2D (Gargano Mortality Study, GMS).

**Materials and methods:** Serum resistin was associated to incident major cardiovascular events in 350 patients with T2D and coronary artery disease from the GHS. Over a follow-up of 1,108 person-year, 33 cardiovascular deaths, 3 non fatal MIs and 8 non fatal strokes occurred (annual incidence rate=0.04). Predicted risk probabilities were derived from the estimated multivariate Cox regression models considered as established risk models for cardiovascular event in diabetic patients. Survival C statistics, Integrated Discrimination Improvement (IDI) and continuous Net Reclassification Index (cNRI) tests were used to evaluate the incremental prognostic information of serum resistin for major cardiovascular events. The time horizon of risk prediction was set to two years. Serum resistin and all-cause mortality were prospectively evaluated in 783 patients from the Gargano Mortality Study (GMS). Over a follow-up of 5,659 person-year, 150 deaths occurred (annual incidence rate=0.027).

**Results:** Aim 1. As previously described, in multivariable-adjusted analysis, serum resistin was strongly associated with incident major cardiovascular events ( $P = 0.001$ ). At two year follow-up, the addition of serum resistin to the most well established model in T2D including age, sex, smoking habits, HbA<sub>1c</sub> and diabetes duration increased the C-statistic from 0.75 (95% CI: 0.65 to 0.85) to 0.80 (95% CI: 0.73 to 0.87)  $P = 0.037$  and showed an IDI of 2%

( $P=0.05$ ). Finally, the addition of serum resistin allowed to reclassify correctly 139 of the 350 patients ( $P=0.002$ ); 15 (33.1%) and 124 (40.7%) in those with and without incident events, respectively. Aim 2. Serum resistin predicted all-cause mortality with HR per SD increment of 1.14, 95% CI: 1.05–1.22;  $P=0.001$  after adjusting for age, sex, smoking habits, HbA1c and diabetes duration.

**Conclusion:** High serum resistin i) is a strong and independent predictor of incident major cardiovascular events and all-cause mortality in T2D; ii) improves CVD risk stratification based on the most frequently used traditional risk factors. Further studies are needed to confirm the potentially important role of resistin as a novel marker to be implemented in the clinical set for predicting CVD in patients with T2D.

Supported by: EFSD/Pfizer grant

## 137

### N-terminal pro-brain natriuretic peptide and cardiovascular mortality in type 2 diabetes: the Casale Monferrato survey

A. Landi<sup>1</sup>, G. Gruden<sup>1</sup>, G. Ghezzo<sup>2</sup>, C. Baldin<sup>2</sup>, C. Amione<sup>1</sup>, A. Schimmenti<sup>1</sup>, L. Spadafora<sup>1</sup>, T. Prinzi<sup>1</sup>, P. Cavallo-Perin<sup>1</sup>, G. Bruno<sup>1</sup>;

<sup>1</sup>Dept. Internal Medicine, Torino, <sup>2</sup>Santo Spirito Hospital, Casale Monferrato, Italy.

**Background and aims:** Circulating levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), a marker of acute heart failure, are associated with increased risk of cardiovascular disease (CVD) in the general population. There is little information on the potential role of NT-proBNP as a biomarker of cardiovascular mortality in diabetic patients and a role of inflammatory markers on this increase has been recently suggested. The aim of this study were to determine: 1) to what extent NT-proBNP values in type 2 diabetic people influences 5-year all causes and cardiovascular mortality, independently on AER and other cardiovascular risk factors; 2) the potential role of CRP on this association.

**Materials and methods:** In the Casale Monferrato Study centralized measurements of NT-proBNP values were available in 1,825 (56.1%) persons out of a population-based cohort of 3,249 persons with diabetes in 2000. They were identified by using independent sources of identification, obtaining an estimated completeness of ascertainment 80%. All measurements were centralized. The independent role of NT-proBNP as predictor of all-cause and cardiovascular mortality in the follow-up period 2000–2006 was assessed with multivariate Cox proportional hazards modeling.

**Results:** Data are based on 390 deaths in 9,101 person-years of observations. A significantly increased mortality risk by quartiles of NT-proBNP was evident ( $p$  for trend  $<0.001$ ), even in people with values in the third quartile. With respect to subjects with NT-proBNP in the lowest quartile ( $<40$  pg/ml), those with values  $>200$  pg/ml had an hazard ratio (HR)=3.05 (95% CI 1.89–4.91) of all-cause mortality and HR=6.99 (95% CI 2.63–18.61) of cardiovascular mortality, after multiple adjustments (Table 1). The inclusion of CRP did not modified results. NT-proBNP has a stronger association with mortality than AER. In people with no CVD at baseline, HR for cardiovascular mortality in the second and in the third quartiles were 3.82 (1.24–13.75) and 3.14 (1.00–9.94).

**Conclusions:** our population-based study indicates that NT-proBNP is a strong independent predictor of short-term mortality risk in type 2 diabetic people, even in those without CVD at baseline. The association between NT-proBNP and mortality is stronger than that provided by AER, is not modified by CRP and is evident even in people with slightly increased values, suggesting its role as an early marker of vascular abnormalities.

	All-cause mortality			Cardiovascular mortality		
	HRs Model 1	HRs Model 2	HRs Model 3	HRs Model 1	HRs Model 2	HRs Model 3
<b>NTproBNP</b>						
<b>0 - 40</b>	1.00	1.00	1.00	1.00	1.00	1.00
<b>41 - 90</b>	1.50 (0.98-2.29)	1.15 (0.68-1.94)	1.17 (0.69-1.97)	2.02 (0.88-4.65)	1.44 (0.46-4.44)	1.45 (0.47-3.49)
<b>91-200</b>	2.12 (1.41-3.18)	1.65 (1.00-2.71)	1.64 (1.00-2.70)	4.31 (2.00-9.30)	3.91 (1.45-10.55)	3.92 (1.45-10.58)
<b>&gt; 200</b>	4.72 (3.22-6.92)	3.11 (1.93-5.01)	3.05 (1.89-4.91)	10.10 (4.80-21.27)	6.96 (2.62-18.52)	6.99 (2.63-18.61)
Table of Contents						
P for trend	$<0.0001$	$<0.0001$	$<0.0011$	$<0.0001$	$<0.0001$	$<0.0001$

P for trend

Model 1: adjusted for age, sex, diabetes duration,

Model 2: plus hypertension, apob/apoA1, HbA1c, BMI, smoke, CVD, AER, eGFR, diabetes treatment

Model 3: plus PCR

## 138

### NT-proBNP and long-term all-cause mortality in a cohort of patients with type 1 diabetes

S.S. Diemar<sup>1,2</sup>, A.-S. Sejling<sup>1</sup>, U. Pedersen-Bjergaard<sup>1</sup>, C. Kistorp<sup>3</sup>, J. Faber<sup>3</sup>, B. Thorsteinsson<sup>1,2</sup>;

<sup>1</sup>Department of Cardiology, Nephrology and Endocrinology, Hillerød

Hospital, Hillerød, <sup>2</sup>Faculty of Health Sciences, University of Copenhagen,

<sup>3</sup>Department of Endocrinology, Herlev Hospital, Denmark.

**Background and aims:** Elevated levels of NT-proBNP are associated with increased cardiovascular mortality and excess all-cause mortality in type 1 diabetes with overt nephropathy and in type 2 diabetes independent of urinary albumin excretion. We assessed the relationship between NT-proBNP and all-cause mortality in a cohort of type 1 diabetes with different degrees of albuminuria.

**Materials and methods:** A cohort of 265 patients with type 1 diabetes was followed for 12 years with recording of all-cause mortality. At baseline NT-proBNP was measured and late diabetic complications were recorded. One patient was excluded due to missing NT-proBNP. Of the remaining 264 patients (59% men, age  $45 \pm 13$  years (mean  $\pm$  SD), and duration of diabetes  $21 \pm 12$  years at entry) 25% had renal disease, ranging from microalbuminuria to elevated serum creatinine, and 8% had known macrovascular disease. NT-proBNP levels were categorised in quartiles. Survival data was calculated and Kaplan-Meier survival and Cox regression analyses were performed.

**Results:** The 12-year all-cause mortality was 15% (39 patients). Excess all-cause mortality was associated with the upper NT-proBNP quartile ( $p<0.001$ ) with a relative risk (RR) of 9.1 [95% CI 4.5–18] ( $p=0.003$ ), presence of renal disease ( $p<0.001$ ) and macrovascular complications ( $p<0.001$ ), and male gender ( $p=0.036$ ) but not with smoking ( $p=0.17$ ). When excluding patients with renal disease ( $n=83$ ), mortality in the upper NT-proBNP quartile was increased ( $p<0.001$ ) with no difference between genders ( $p=0.88$ ). When excluding both patients with renal disease and any macrovascular events ( $n=93$ ), belonging to the upper NT-proBNP quartile was still associated with increased all-cause mortality (RR 6.5 [2.1–21] ( $p=0.003$ )), with no difference between genders ( $p=0.34$ ).

**Conclusion:** Elevated levels of NT-proBNP are associated with excess all-cause mortality in type 1 diabetes even when excluding patients with renal and macrovascular disease.



## OP 24 The transcriptome of healthy and stressed beta cells

139

### Quantitative analysis of the human beta cell transcriptome

A.C. Nica, H. Ongen, J.-C. Irminger, S.E. Antonarakis, P.A. Halban, E.T. Dermitzakis;  
Department of Genetic Medicine and Development, University of Geneva, Switzerland.

**Background and aims:** Gene expression shapes phenotypic differences among cell-types, individuals and populations. Transcript abundance provides a direct link between genotype and organismal phenotypes, helping thus to interpret selected disease-predisposing loci. As most phenotypes manifest themselves only in certain tissues, mRNA profiling is most informative in the context of trait-relevant cell-types. Towards this end, we provide here the first detailed description of the human beta cell transcriptome using deep coverage RNA sequencing (RNA-seq) and perform the first comparison of beta cell - islet expression profiles.

**Materials and methods:** Islets were obtained from 11 cadaveric pancreata from individuals without documented diabetes. Islet cells were sorted by FACS to obtain “beta cell” (approx. 90% pure) and also (from 6 preparations) beta-cell depleted “non-beta cell” populations. RNA was extracted and each sample was sequenced in one Illumina HiSeq lane, attaining thus an unprecedented transcriptome resolution (up to 200 million quality filtered reads per sample). Additionally, we sequenced islet DNA from 7 donors at 13 × depth, performed variant calling and detected allele specific effects on expression.

**Results:** As expected, insulin (INS) was by far the most abundantly transcribed gene, followed by INS-IGF2 and IGF2, making up ~ 39%, 9% and 3% of the total nuclear beta cell transcriptome respectively. While the relative percentages are lower, this ranking is maintained in the islet (INS 27%, INS-IGF2 5%, IGF2 1.6%). The ranked correlation between all beta cell and islet-expressed genes is high (Spearman rho = 0.934) and we estimate that 85% of the variance in beta cell gene expression can be explained by using islet expression as proxy. A principal component analysis confirms that beta cell and islet preparations markedly separate from non-beta cells, with insulin, IGF-2, glucagon, transthyretin, pancreatic polypeptide and somatostatin driving most of this separation. We notice a clear clustering of the islet derived RNA-seq data in the context of 20 other tissues, with thyroid, ovary and brain as highest correlating tissue types. Most diabetes susceptibility genes (80% of T1D, 92% of T2D) are expressed in the beta cell, corroborating the cell-type’s essential role in the etiology of the disease. We further characterize the transcriptome by detecting alternative splicing events (defined as changes in frequency of exon pairings) and identifying novel exon junctions. In each sample we observe significant allelic differences in transcript levels at a subset of heterozygous sites, some of which map to diabetes genes or genes for which expression QTLs have been detected in other tissues.

**Conclusion:** In this study we quantify all genes expressed in adult human beta cells, catalogue alternative splicing and allele specific expression events and report the first beta cell - islet expression profile comparison. We thereby provide a valuable resource to the community, aiding our understanding of the genetics of both type 1 and type 2 diabetes.

*Supported by: JDRF Grant 17-2011-284*

140

### Small-Maf factors are involved in the regulation of pancreatic beta cell function in vivo

T. Kondo, H. Nomoto, N. Fujimori, H. Miyoshi, T. Atsumi;  
Medicine II (Immunology and Metabolism), Hokkaido University, Sapporo, Japan.

**Background and aims:** The Maf family, which belongs to leucine zipper transcription factors, consists of two groups, large-Maf and small-Maf factors. MafA, one of the major regulators of insulin gene expression and beta-cell function, is a member of the large-Maf factors and positively regulates insulin gene expression. Our previous study demonstrated small-Maf factors (MafF, MafG and MafK), that lack an N-terminal transactivation domain present in the large-Maf factors, are also expressed in beta cells and thus, can function as a negative regulator. We also showed that both oxidative stress and lipotoxicity reduce insulin gene expression and concomitantly increase both small-

Maf mRNA and protein expressions in a beta-cell line. Here, we evaluated the effect of small-Maf factors on glucose tolerance in vivo.

**Materials and methods:** Three lines of transgenic (Tg) mice with beta-cell specific expression of dominant-negative small-Maf factor (DN-MafK) were established on the C57BL/6J background. All lines of Tg mice showed normal size and growth. Both wild type (Wt) and Tg male mice were fed a regular-chow (R) or high-fat (HF) diet respectively from 6 weeks of age. At 20 weeks, glucose tolerance test was performed and then islets were isolated and analyzed to determine expressions of MafA, small-Mafs and other beta cell-related molecules by real-time RT-PCR and western blot analyses. Pancreatic sections were obtained for immunohistochemistry to evaluate the beta-cell mass and those expressions.

**Results:** There were no significant differences in body weight and food intake between Wt and Tg mice. Feeding HF diet resulted in higher levels of serum insulin and blood glucose, and increased beta-cell mass. Small-Maf and MafA protein expressions were significantly increased. Interestingly, despite similar MafA expression pattern, glucose tolerance in HF diet-fed Tg (HFD-Tg) mice was significantly improved as compared with HF diet-fed Wt (HFD-Wt) mice. HFD-Tg mice showed higher serum insulin level after a glucose load than HFD-Wt mice.

**Conclusion:** Not only MafA but small-Maf factors are important for the regulation of insulin expression in vivo. Inhibition of small-Maf factors in beta cells leads to increment of capability to secrete insulin on demand. Therefore, preventing small-Maf function in beta cells may represent a novel therapeutic target to improve beta-cell function.

141

### Pancreatic beta cell dedifferentiation into neurogenin3-positive progenitor cells and conversion into non-beta endocrine cells as mechanisms of diabetic beta cell failure

C. Talchai, D. Accili;  
Medicine, Columbia University, New York, USA.

Diabetes is associated with  $\beta$ -cell dysfunction. But it remains unclear whether the latter results from reduced  $\beta$ -cell number or function. We have previously shown that transcription factor FoxO1 integrates  $\beta$ -cell proliferation with adaptive changes during metabolic stress, such as the hyperglycemia of diabetes. Thus, FoxO1 haploinsufficiency is associated with increased  $\beta$ -cell proliferation, while FoxO1 gain-of-function commonly occurs in response to hyperglycemia-dependent oxidative stress, and causes an arrest of  $\beta$ -cell proliferation and a reduced metabolic activity that we have termed “metabolic diapause”. We interrogated the contribution of these two processes (proliferation vs. function) to  $\beta$ -cell dysfunction, using mice lacking FoxO1 in  $\beta$ -cells. FoxO1 ablation caused reduced  $\beta$ -cell mass and hyperglycemia following physiologic (multiparity, aging) and pathophysiologic challenges (chemical  $\beta$ -cell ablation by the toxin streptozotocin, insulin resistance). Surprisingly, lineage-tracing experiments demonstrated that loss of  $\beta$ -cell mass in FoxO1 ablation was due to  $\beta$ -cell dedifferentiation (i.e. loss of  $\beta$ -cell maturation factors such as Pdx1, MafA and Glut2), and not cell death. Dedifferentiated  $\beta$ -cells “regress” to a progenitor-like stage characterized by expression of ChromograninA, and increased levels of Neurogenin3, L-myc, and Hdac1. Moreover, similar lineage-tracing experiments with marker of endocrine non- $\beta$  cells ( $\alpha$ ,  $\delta$ , and Pp cells) indicated that FoxO1-deficient dedifferentiated  $\beta$ -cells partly converted into non- $\beta$  cells, supporting that they retained differentiation potentials, and consequently giving rise to hyperglucagonemia. This is of great interest, in that diabetic patients are characterized by inappropriate hyperglucagonemia that contributes to their disease status. Strikingly, we identify the same sequel of events and dedifferentiation as a common feature of different models of murine diabetes. Our findings indicate that in response to pathophysiological stresses,  $\beta$ -cell differentiation is not terminal but requires FoxO1 to enforce  $\beta$ -cell fate and prevent  $\beta$ -cell conversion. We propose that dedifferentiation trumps endocrine cell death in the natural history of  $\beta$ -cell failure, and suggest that treatment of  $\beta$ -cell dysfunction should aim at restoring  $\beta$ -cell differentiation, rather than increasing  $\beta$ -cell replication.

*Supported by: NIH DK58282*

## 142

**Effects of palmitate treatment on global gene expression in human pancreatic islets**E. Hall<sup>1</sup>, P. Volkov<sup>1</sup>, T. Dayeh<sup>1</sup>, M. Dekker Nitert<sup>2</sup>, C. Ling<sup>1</sup>;<sup>1</sup>Epigenetics & Diabetes, Clinical sciences, Malmö, Sweden, <sup>2</sup>Royal Brisbane Clinical School, Herston, Australia.

**Background and aims:** Chronic exposure to high levels of fatty acids have a negative effect on the beta cell function and hence on insulin secretion. Elevated plasma levels of free fatty acids are often seen in patients with type 2 diabetes and in obese individuals. However, different types of fatty acids can have different effects on pancreatic islets and beta cells. In particular, the long chain saturated fatty acids, such as palmitate and stearate, seem to have a cytotoxic effect. Previous studies in isolated pancreatic islets from rat and mouse have shown that, a long term treatment with palmitate inhibits the glucose stimulated insulin secretion. Additionally, a genome wide expression analysis on MIN6 beta cells revealed differences in the gene expression pattern in cells treated with palmitate compared with control. In this study, we aim to investigate if palmitate treatment for 48 hours affects the genome-wide mRNA expression pattern and glucose-stimulated insulin secretion in human pancreatic islets.

**Materials and methods:** Pancreatic islets from 15 deceased human donors were cultured in control media or palmitate media for 48h. After the 48h incubation, RNA and DNA were extracted. Global gene expression data was obtained using the GeneChip® Human Gene 1.0 ST Array (Affymetrix). The expression data was normalized using the RMA method, and a Wilcoxon signed rank test was performed to detect differences between groups. The results were corrected for multiple testing using FDR analysis and a  $q$  value  $< 0.05$  was considered as significant. Finally, pathway analyses were conducted using the on-line tool Webgestalt. Glucose-stimulated insulin secretion at 16.7 mM glucose was analyzed from 5 replicates of 10 islets per culture condition. **Results:** We identified 1860 genes that were differentially expressed at  $q$  value  $< 0.05$  in human islets exposed to palmitate. Out of these, 1230 genes were down regulated and 630 genes were up regulated due to palmitate treatment. While the down regulated genes included several potassium channels that may affect insulin secretion, several up regulated genes were involved in inflammation. Of the significantly down regulated genes, 1188 had a unique Entrez ID and these were used for pathway analysis. 42 pathways had a  $q < 0.05$  and among the significant pathways we found several pathways involved in metabolism, for example fatty acid metabolism. Out of the significantly up regulated genes, 600 had a unique Entrez ID and these were used for pathway analysis. 6 pathways had a  $q < 0.05$  and among these were for example the PPAR signalling pathway. Finally, glucose-stimulated insulin secretion was impaired in human islets exposed to palmitate compared to control media ( $1.11 \pm 0.07$  vs.  $1.32 \pm 0.08$ ,  $p$ -value 0.046).

**Conclusion:** Our study has identified individual genes and metabolic pathways that are regulated by lipotoxicity in human pancreatic islets. Differential expression of these genes may lead to impaired insulin secretion.

*Supported by: Swedish Research Council, ALF, EFSD/Lilly grant, Söderberg & Pahlsson foundation*

## 143

**Bach1 deficiency protects pancreatic beta cells from oxidative stress injury**K. Kondo<sup>1</sup>, Y. Ishigaki<sup>1</sup>, J. Gao<sup>2</sup>, T. Yamada<sup>2</sup>, J. Imai<sup>1</sup>, S. Sawada<sup>1</sup>, A. Muto<sup>3</sup>, Y. Oka<sup>1</sup>, K. Igarashi<sup>3</sup>, H. Katagiri<sup>2</sup>;<sup>1</sup>Division of molecular metabolism and diabetes, <sup>2</sup>Department of Metabolic Diseases, Center for Metabolic Diseases, <sup>3</sup>Department of Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan.

**Background and aims:** BTB and CNC homology 1 (Bach1) is a transcriptional repressor of anti-oxidative enzymes, such as heme oxygenase-1 (HO-1), an inducible enzyme that protects cells in various models of organ injuries. Under oxidative stress, Bach1 loses its DNA-binding activity resulting in activation of transcriptions of oxidative stress-responsive genes. In Bach1-deficient mice, HO-1 is constitutively expressed at higher levels in many tissues, indicating that Bach1 plays a major role in negative regulation of HO-1 expression. A number of previous studies have revealed that a wide range of pathological conditions, including myocardial infarction, atherosclerosis, steatohepatitis, intestine injury, lung injury and neural tissue damage, were prevented in Bach1-deficient mice. Apart from Bach1, induction of HO-1 is known to protect pancreatic  $\beta$ -cells from oxidative injury, and to improve

insulin sensitivity in certain models, such as obese rodents. These findings prompted us to hypothesize that ablation of Bach1 is an encouraging approach to prevent the development of diabetes.

**Materials and methods:** Glucose metabolism in high-fat diet (HFD)-induced insulin resistance and alloxan-induced pancreatic  $\beta$ -cell loss was studied using C57BL/6J wild-type (WT) and homozygous Bach1-deficient C57BL/6J mice. HFD was administered from 6 to 12 weeks of age, and alloxan was injected 50mg/kg to 8 week-old normal chow diet mice. Real-Time PCR and TUNEL staining of the pancreatic islets were performed for further evaluation.

**Results:** The expressions of HO-1 in the liver, white adipose tissue and pancreatic islets were up-regulated 6 to 30 fold in Bach1-deficient mice. In HFD treated mice, no differences were observed in body weight gain, glucose tolerance and in insulin sensitivity between WT and Bach1-deficient mice. In alloxan treated mice, the reduction in pancreatic insulin content was only to 83% in Bach1 deficient mice, while in WT mice, the insulin content was reduced to 46%. In this alloxan model, TUNEL-positive cells in the pancreatic islets of Bach1-deficient mice were markedly decreased, by 60%, compared with those in WT mice.

**Conclusion:** Bach1 has minimal roles in the development of obesity and insulin resistance. Bach1 deficiency-induced HO-1 enhancement has a beneficial impact on oxidative stress-induced  $\beta$ -cell injury.

## 144

**A glucose-regulated cell cycle gene module is selectively lost in pancreatic islets during aging**A. Moreno-Asso<sup>1,2</sup>, A. Grilli<sup>3</sup>, C. Castaño<sup>1,2</sup>, N. Lopez-Bigas<sup>3</sup>, A. Novials<sup>1,2</sup>, J.-M. Servitja<sup>1,2</sup>;<sup>1</sup>Diabetes and Obesity Laboratory, IDIBAPS, <sup>2</sup>CIBERDEM, <sup>3</sup>Biomedical Genomics Group, Universitat Pompeu Fabra, Barcelona Biomedical Research Park (PRBB), Barcelona, Spain.

**Background and aims:**  $\beta$ -cell fate is determined by tightly regulated transcriptional networks that are modulated by extracellular stimuli to allow  $\beta$  cells to adapt to insulin demands. Besides its role as an insulin-secretagogue, glucose is also a potent regulator of gene expression in  $\beta$  cells. Aging is a major risk factor for the development of type 2 diabetes, but the exact contribution of  $\beta$  cells in this process and their capacity to adapt to increased glucose concentrations during aging is still poorly understood. We sought to decipher the glucose-dependent gene modules in mouse pancreatic islets and how this adaptative transcriptional response is altered in aging.

**Materials and methods:** Pancreatic islets from young (6 weeks) and old ( $> 50$  weeks) mice were isolated and cultured at different glucose concentrations. Islet transcriptional profiling was performed by Affymetrix gene expression microarrays and quantitative RT-PCR. Gtools (www.gtools.org) and Gene Set Enrichment Analysis were used to uncover glucose-regulated gene modules.

**Results:** The transcriptional profile of pancreatic islets cultured at 11 mM, the usual in vitro concentration, was the closest to that of fresh islets, indicating that glucose-elicited cues are involved in maintaining the normal  $\beta$ -cell transcriptional program. At severe hypoglycemia (3 mM), the expression of over 15% of islet genes was altered compared to the 11 mM condition. At a basal glucose concentration (5.5 mM), one third of these genes exhibited a similar behavior, but the rest were not affected, indicating that the latter are altered only at severe hypoglycemia. Low glucose levels very rapidly induced the expression of ubiquitous stress genes and nutrient sensors, as well as genes involved in mitochondria biogenesis. The expression of most of these genes rapidly returned to normal levels upon reestablishment of normal glucose levels. By contrast, high glucose concentrations maintained the expression of genes with a more restricted expression pattern such as those involved in  $\beta$ -cell fate acquisition or co-expressed in the nervous system. Of interest, a cell cycle gene module was the most enriched among glucose-induced genes. These genes were already differentially expressed between basal (5 mM) and high (11 mM) glucose levels, suggesting that they may be regulated within the physiological range of glycemia. Unexpectedly, expression profiles of islets from old mice at different glucose concentrations broadly maintained the same profile observed in young islets, except the cell cycle gene module that was selectively lost during aging.

**Conclusion:** Global expression profiling in pancreatic islets has uncovered gene modules regulated by glucose that are likely to be involved in the adaptation of  $\beta$  cells to the nutritional demands of the organism. Although old islets broadly maintained the same transcriptional response to glucose observed in young islets, the regulation of a cell cycle gene module was selectively lost.

This indicates that old  $\beta$  cells maintain most of the glucose-dependent signaling and transcriptional networks except their capacity to proliferate in close correlation to glucose levels.

Supported by: EFSD/Lilly fellowship, MICINN BFU2010-17639, CIBERDEM

## OP 25 Intervention studies in type 1 diabetes

### 145

#### Recent data from DIA-AID 1, a global phase III clinical study using DiaPep277 for the treatment of newly diagnosed type 1 diabetes patients

I. Raz<sup>1</sup>, T. Linn<sup>2</sup>, A.-G. Ziegler<sup>3</sup>, G. Scherthanner<sup>4</sup>, F. Bonnici<sup>5</sup>, R. Eren<sup>6</sup>, D. Elias<sup>6</sup>, S. Dagan<sup>6</sup>, P. Pozzilli<sup>7</sup>, On behalf of the DIA-AID 1 Study Group; <sup>1</sup>Diabetes Unit, Department of Medicine, Hadassah University Hospital, Jerusalem, Israel, <sup>2</sup>Universitätsklinikum Giessen, Germany, <sup>3</sup>Institut für Diabetesforschung an der Klinik und Hochschulambulanz für Kinder, München, Germany, <sup>4</sup>Rudolfstiftung Hospital-Vienna, Austria, <sup>5</sup>New Groote Schuur Hospital, Cape Town, South Africa, <sup>6</sup>Andromeda Biotech Ltd., Yavne, Israel, <sup>7</sup>Universita Campus Bio-Medico, Rome, Italy.

**Background and aims:** DiaPep277, a peptide derived from the human heat shock protein 60, immunomodulates the immune system inducing T regulatory cells that prevents destruction and preserves beta-cell function in type 1 diabetes (T1D). DIA-AID 1, a phase III clinical study, was conducted to evaluate the safety and efficacy of DiaPep277 in newly diagnosed T1D patients.

**Materials and methods:** A randomized, double-blinded, placebo-controlled study included patients in the age range of 16–45, not more than 3 months after diagnosis of T1D, a fasting C-peptide > 0.2 nmol/L, and positive islet auto-antibodies. Subjects received 1 mg of DiaPep277 or placebo at 0, 1, 3, 6, 9, 12, 15, 18, 21 months. The efficacy primary endpoint was defined as the change from baseline to end of study in stimulated C-peptide area under curve (AUC) secretion measured by the glucagon stimulated test. Key secondary endpoints included change from baseline to end of study in Mixed-Meal-stimulated C-peptide AUC secretion; proportion of subjects who maintained HbA1c treat-to-target of  $\leq 7\%$  at end of the study, and change from baseline to endpoint in basal fasting C-peptide.

**Results:** 457 patients were randomized. The target population for efficacy included subjects who entered the study according to the major inclusion/exclusion criteria, received at least 1 dose of DiaPep277, and have at least one measurement post baseline (MITT population, n = 422). Significant preservation of C-peptide levels in patients treated with DiaPep277 was demonstrated. The decline in C-peptide AUC from baseline to study end was -3.1 nmol/L in the treated arm compared to -4.1 nmol/L in the placebo, relative treatment effect of 24 %, p=0.037. This preservation was even more significant in patients who completed two years of therapy in full compliance with the study protocol (29% relative change, p=0.011). Furthermore, clinical outcomes support the preservation of beta cells observed by the levels of C-peptide. The percentage of patients who maintained HbA1c levels  $\leq 7\%$  at study end was significantly higher in the DiaPep277 treated group as compared to placebo (56% vs 44%, p= 0.035). Similarly, the proportion of patients who required an insulin dose of less than 0.5 U/K/Day at study end was significantly higher in the treated arm, 59% vs 46%, p=0.0232. Treated patients experienced fewer hypoglycemic events per month, 69 in the treated arm compared to 83 in the placebo. DiaPep277 was well tolerated. No significant differences in drug-related adverse events were reported between the treatment and placebo groups. Additional data are currently being evaluated.

**Conclusion:** DIA-AID 1 met the primary and key secondary endpoints. Patients treated with DiaPep277 preserved their beta-cell function and were better controlled compared to patients on placebo. A second confirmatory phase III clinical study, DIA-AID 2 is currently being conducted worldwide in newly diagnosed adult T1D patients.

Clinical Trial Registration Number: NCT006 15 264

### 146

#### Teplizumab preserves C-peptide in subjects with type 1 diabetes: 2-year results from the Protégé Study

J. Ludvigsson<sup>1</sup>, W. Hagopian<sup>2</sup>, D. Carlin<sup>3</sup>, K.L. King<sup>3</sup>, S. Johnson<sup>3</sup>, E. Bonvini<sup>3</sup>, S. Koenig<sup>3</sup>, A.G. Daifotis<sup>3</sup>;

<sup>1</sup>Div of Pediatrics, Dept of Clin Exp Med, Linköping University, Linköping, Sweden, <sup>2</sup>Pacific Northwest Diabetes Research Institute, Seattle, <sup>3</sup>MacroGenics, Rockville, USA.

**Background and aims:** Teplizumab is an Fc modified, monoclonal antibody that targets the CD3 T-cell receptor (TCR) complex. The Protégé study failed its primary endpoint at 1 year, but showed results in other endpoints, such as



C-peptide, particularly in certain subgroups that suggested a biologic effect. We now report results at 2 years.

**Materials and methods:** In a global, multicenter, double-blind study, 516 subjects with new onset (<12 weeks) T1D were randomized and treated at baseline and 6 months: A. full 14-day regimen; B. 1/3 14-day regimen; C. full 6-day regimen; or D. placebo, and were followed for 24 months. Endpoints included a composite of HbA<sub>1c</sub> <6.5% and insulin <0.5 U/kg/day (primary at 1 year), C-peptide (secondary at 1 and 2 years) by mixed meal tolerance test (MMTT), and adverse events.

**Results:** 513 subjects were dosed: (US 158, India 142, Europe 179, others 34). The full 14-day regimen better preserved C-peptide compared to placebo at 2 years. At study day 140 and months 12, 18 and 24, teplizumab limited the decline in C-peptide in subjects with early T1D. The effect was greatest in subjects <18 years, US subjects, subjects treated ≤6 weeks after diagnosis, and subjects with baseline C-peptide AUC >0.2 nmol/L/min (Table 1). In adults, the rate of C-peptide decline was slower than in younger subjects, and the treated group did not have as great an improvement.

Table 1: Least Square Mean C-peptide Change from Baseline<sup>1</sup> at 2 Years

Group	Full 14-Day Regimen		Placebo		p-value <sup>2</sup>
	N	Mean ± SEM	N	Mean ± SEM	
All Subjects	131	-0.13 ± 0.01	64	-0.19 ± 0.02	0.027
≤6 Weeks	34	-0.07 ± 0.03	14	-0.21 ± 0.04	0.006
USA	30	-0.17 ± 0.03	14	-0.29 ± 0.04	0.010
All with Baseline C-peptide >0.2 nmol/L	112	-0.15 ± 0.02	57	-0.21 ± 0.02	0.036
8–17 years	69	-0.16 ± 0.02	32	-0.23 ± 0.03	0.031
18–35 years	62	-0.11 ± 0.02	32	-0.14 ± 0.03	0.280

<sup>1</sup> Based on least square means obtained from mixed model repeated measures analyses

<sup>2</sup> Unadjusted for multiple comparisons

Subjects who received the full 14-day regimen tended to use less insulin while achieving an HbA<sub>1c</sub> <7% (effect strongest with baseline C-peptide >0.2 nmol/L/min). No differences across groups were observed in the composite endpoint of insulin <0.5 U/kg/day and HbA<sub>1c</sub> <6.5%. Adverse events were more common in teplizumab full 14-day regimen versus placebo, including: mechanism related leukopenia (73% vs 38%) and lymphopenia (86% vs 26%), decreases in neutrophils (47% vs 31%) and eosinophils (6% vs 0%), increased alanine aminotransferase (34% vs 17%) and bilirubin (22% vs 12%). All were transient. Other treatment-related AEs were transient rash (54% vs 20%), pruritus (16% vs 4%), nausea (20% vs 12%), vomiting (15% vs 6%), and chills (10% vs 2%). CRS in ~6% of teplizumab subjects, was managed with antipyretics and antihistamines. No difference was seen in pyrexia, myalgias, arthralgias, overall infections, or SAEs (~12%).

**Conclusion:** The 2-year Protégé results are consistent with those reported at 1 year. Two 14-day courses of teplizumab preserved insulin secretion in early T1D (<12 weeks), especially in subjects with very recent-onset (≤6 weeks) T1D, residual C-peptide (>0.2 nmol/L/min), aged 8–18 years, and in a US population, with an acceptable safety profile.

Clinical Trial Registration Number: NCT00385697

## 147

### Alpha-1 antitrypsin therapy in new-onset type 1 diabetes: interim results from Part I of the RETAIN study

M.R. Ehlers<sup>1</sup>, P.A. Gottlieb<sup>2</sup>, K. Herold<sup>3</sup>, M.R. Rigby<sup>4</sup>, S.M. Willi<sup>5</sup>, S. Aggarwal<sup>6</sup>, D. Phippard<sup>6</sup>, D.M. Gelmont<sup>7</sup>, B. Jepsen<sup>8</sup>, J. McNamara<sup>9</sup>, T. Strom<sup>10</sup>, G.C. Weir<sup>11</sup>, RETAIN Study Team;

<sup>1</sup>Clinical Trials Group, Immune Tolerance Network, San Francisco,

<sup>2</sup>Pediatrics, Barbara Davis Center, Aurora, <sup>3</sup>Immunobiology, Yale University, New Haven, <sup>4</sup>Pediatrics, Indiana University, Indianapolis, <sup>5</sup>Pediatrics, Children's Hospital of Philadelphia, Philadelphia, <sup>6</sup>Biomarker & Discovery Research, Immune Tolerance Network, Bethesda, <sup>7</sup>Baxter Healthcare Corporation, Westlake Village, <sup>8</sup>Biostatistics, RhoFed, Inc., Chapel Hill, <sup>9</sup>DAIT, National Institute of Allergy and Infectious Diseases, Bethesda,

<sup>10</sup>Medicine, Beth Israel Deaconess Medical Center, Boston, <sup>11</sup>Joslin Diabetes Center, Boston, USA.

**Background and aims:** Immune modulation in new-onset type 1 diabetes (T1D) has shown some success in clinical trials, but durable disease remis-

sion has not been achieved. Treatment with alpha-1 antitrypsin (AAT) reverses diabetes in the NOD mouse with induction of immune tolerance, and prevents rejection in islet allograft models. In the mouse, AAT appears to be broadly anti-inflammatory and has direct anti-apoptotic effects on beta cells. The RETAIN study is a phase II trial in new-onset T1D patients evaluating the ability of AAT to preserve beta-cell function, and here we report preliminary results from Part I.

**Materials and methods:** Part I of RETAIN is a multicenter, open-label, dose-escalation, safety and pharmacokinetic (PK) study in which autoantibody-positive T1D subjects within 100 days of diagnosis received 6 weekly i.v. infusions of human plasma-derived AAT at 45 mg/kg, followed by a 3-week washout, and then 6 infusions at 90 mg/kg; subjects were divided into an older (16–35 years) and a younger (8–15 years) cohort. Endpoints included safety, PK, and metabolic assessments. Intensive PK sampling was performed during and after infusions 1, 6, 7, and 12. Endogenous insulin secretion was assessed by 2-hour C-peptide AUC in response to a mixed-meal tolerance test at baseline and week 18.

**Results:** 16 subjects were enrolled in the trial and received study drug. All except one completed ≥ 80% of planned AAT infusions; 1 subject discontinued due to a suspected drug rash (grade 2). There were no SAEs and the infusions were generally well tolerated. Mean (± SD) baseline (endogenous) and peak plasma AAT levels after the 6<sup>th</sup> low-dose and high-dose infusions, respectively, were 1.07 ± 0.13, 2.20 ± 0.10, and 3.22 ± 0.33 g/L in the older cohort and 1.20 ± 0.11, 2.11 ± 0.21, and 3.42 ± 0.63 g/L in the younger cohort. Peak AAT levels generally declined by 50% at 48 hours post-infusion, but mean plasma AAT levels remained 50% above endogenous levels 120 hours after the final high-dose infusion. At baseline vs. week 18, mean (± SD) 2-hour C-peptide AUC levels (both cohorts) were 0.714 ± 0.324 and 0.706 ± 0.333 pmol/mL, respectively, while insulin use changed from 0.186 ± 0.161 to 0.176 ± 0.127 U/kg/day in the older cohort and from 0.385 ± 0.308 to 0.294 ± 0.149 U/kg/day in the younger cohort; HbA<sub>1c</sub> (combined) changed from 8.0 ± 1.7% to 6.3 ± 0.9%.

**Conclusion:** AAT infusions are well tolerated in new-onset T1D subjects. Metabolic parameters are stable and C-peptide secretion is preserved at the early time point (18 weeks). Additional follow-up to 2 years is required to assess durability of the effect. Mechanistic studies are underway to determine how AAT functions in vivo and to develop a relevant biomarker of biologic activity, prior to initiating Part II of RETAIN.

Clinical Trial Registration Number: NCT01183468

Supported by: ITN, NIAID, NIDDK, JDRF

## 148

### Beta cell function in latent autoimmune diabetes in adults treated with linagliptin versus glimepiride: exploratory results from a 2 year double-blind randomised controlled study

O. Johansen<sup>1</sup>, B. Boehm<sup>2</sup>, V. Grill<sup>3</sup>, P.A. Torjesen<sup>4</sup>, S. Bhattacharya<sup>5</sup>, S. Patel<sup>6</sup>, K. Wetzel<sup>7</sup>, H.-J. Woerle<sup>7</sup>;

<sup>1</sup>Boehringer Ingelheim, Asker, Norway, <sup>2</sup>University of Ulm, Germany,

<sup>3</sup>Norwegian University of Science and Technology, Trondheim, Norway,

<sup>4</sup>Aker University Hospital, Oslo, Norway, <sup>5</sup>Boehringer Ingelheim, Biberach,

Germany, <sup>6</sup>Boehringer Ingelheim, Bracknell, UK, <sup>7</sup>Boehringer Ingelheim,

Ingelheim, Germany.

**Background and aims:** Latent autoimmune diabetes in adults (LADA) is associated with a more rapid decline in β-cell function compared to common type 2 diabetes (T2D). Presently, no treatment modality is drug of choice in LADA. We compared the impact of treatment with the DPP4 inhibitor linagliptin (lina) 5mg/d and the sulphonylurea glimepiride (glim) 1–4mg/d on β-cell function in patients retrospectively identified with LADA who had insufficient glycaemic control despite metformin therapy in a 2 year study.

**Materials and methods:** Patients were classified as LADA if one or more of the autoantibodies assessed (GAD65, ICA, IA-2A, IAA) were present at baseline or any on-treatment visit. GAD was assessed using RIA methodology (cut-off 0.05 [sensitivity 82%/specificity 98.89% in the Diabetes Autoantibody Standardization Program 2010]).

**Results:** The study cohort comprised 1519 patients (16 countries), with assumed common T2D. The prevalence of LADA was 7.8% (n=118). GAD65 was the most prevalent autoantibody (6.5%) whereas ICA (0.3%), IA-2A (1.2%) and IAA (0.2%) were rare. Proportion of patients with ≥2 positive antibodies was 0.4%. Baseline characteristics in GAD65+ LADA patients treated with lina (n=65) or glim (n=53) were fairly well balanced (respective age 59/63 years, BMI 30.3/31.7 kg/m<sup>2</sup> and diabetes duration >5 years 62%/59%). C-peptide was available in a subset and as indicated, GAD65+

patients treated with lina preserved C-peptide significantly better than those treated with glim over a 2 year trajectory (Table). HbA1c reductions were of similar magnitude in the groups.

**Conclusion:** In conclusion, treatment with lina or glim in LADA could have differing impacts on long-term  $\beta$ -cell function. Further research exploring the observed potential modulating impact of lina on LADA is ongoing.

**Table:** Changes in fasting C-peptide over time by treatment and GAD65 autoimmune status

	28 weeks		52 weeks		104 weeks	
	Lina GAD+	Glim GAD+	Lina GAD+	Glim GAD+	Lina GAD+	Glim GAD+
n	21	17	14	14	9	9
Baseline C-peptide	821	1326	835	1425	944	1374
Follow-up C-peptide	917	1221	978	1246	1146	1345
$\Delta$ C-peptide	+96****	-105	+143****	-179	+202***	-29
Baseline HbA1c (%)	7.62	7.75	7.50	7.46	7.40	7.40
$\Delta$ HbA1c	-0.25	-0.75	-0.49	-0.52	-0.41	-0.49

\*\*\* $P < 0.001$  versus baseline; \*\* $P < 0.01$  versus glim

Clinical Trial Registration Number: NCT00622284

Supported by: Boehringer Ingelheim

## 149

### Combined pancreas-kidney transplantation in recipients with diabetes end-stage kidney disease improve long-term patient survival

T.G. Jenssen<sup>1,2</sup>, J.-P. Lindahl<sup>1</sup>, T. Leivestad<sup>1</sup>, A. Hartmann<sup>1</sup>

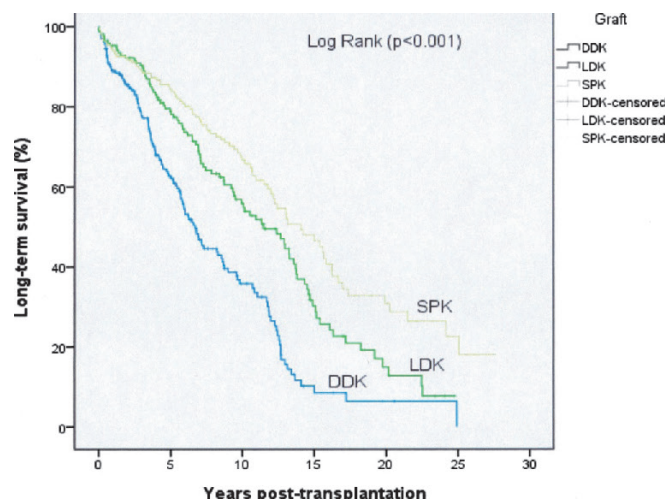
<sup>1</sup>Medicine, Rikshospitalet, Oslo, <sup>2</sup>Department of Clinical Medicine, University of Tromsø, Norway.

**Background and aims:** From 1983 a total of 5885 consecutive patients have received a renal transplant at our national centre. Among these recipients, 629 had end-stage kidney disease due to diabetes mellitus. Traditionally recipients with type 1 diabetes end-stage nephropathy have been offered a simultaneous pancreas and kidney transplantation (SPK) if they were fit, motivated and were below 50–55 years of age. To avoid or reduce time on dialysis recipients with a live kidney donor have generally received a single live donor kidney (LDK) often with the intention of later receiving a pancreas. Due to the increased operative risk of SPK older and co-morbid patients generally have received a single deceased donor kidney (DDK).

**Material and methods:** With this policy the type 1 diabetes patients receiving LDK or SPK are comparable with regard to age and co-morbidity, and differences in outcomes may reflect the metabolic effects of a functioning pancreas transplant. We examined the long-term patient survival of recipients with diabetes end-stage kidney disease grouped by mode of treatment at first transplantation.

**Results:** Patient survival of SPK (n=221), LDK (n=171) and DDK (n=237) is presented in figure 1. Mean follow-up time after transplantation was  $7.1 \pm 5.7$  years. The Kaplan-Meier plots show that survival is superior with combined pancreas-kidney transplantation compared to both LDK and DDK recipients. Actual half-life was 14.1 years for the combination (SPK), 11.5 years for LDK and 6.7 years for DDK recipients. In a Cox regression analysis adjusting for recipient age, we found that receiving a combined graft (SPK) was protective for death compared to both LDK recipients (Hazard Ratio 0.72 (0.54–0.97),  $p=0.029$ ) and DDK recipients (HR 0.50 (0.36–0.68),  $p<0.001$ ). Similarly receiving a single live kidney had a better patient survival compared to receiving a single deceased donor kidney (HR 0.69 (0.52–0.92),  $p=0.013$ ). Ten years cumulative survival proportion was 66.7 % in the SPK population, which we find acceptable in this high risk group.

**Conclusion:** PK recipients have a markedly better long-term survival compared to both DDK and LDK recipients in diabetes end-stage kidney disease. More recipients with type 1 diabetes should be offered a SPK transplantation. Figure 1 Kaplan-Meier plot.



## 150

### Cardiac morphology and function in type 1 diabetic patients with successful pancreas transplant alone

M. Occhipinti, L. Rondinini, R. Mariotti, N. Scelza, M. Barsotti, F. Vistoli, G. Amorese, U. Boggi, P. Marchetti;

Dept. Endocrinology and Metabolism, University of Pisa, Italy.

**Background and aims:** Whereas combined pancreas and kidney transplantation is an established procedure for the treatment of T1D patients with end stage renal disease, the role of pancreas transplant alone (PTA) in the therapy of T1D subjects with preserved kidney function is still matter of discussion. Aim of this study was to investigate the effects of PTA on cardiac morphology and function in T1D subjects with more than three years of post transplantation follow-up (FU).

**Materials and methods:** Eighty-eight T1D patients received PTA in our centre, mainly performed by the enteric portal drainage and standard immunosuppression, and with 5 years recipients and graft survival of 98.7% and 76.8%, respectively. A series of 35 consecutive subjects with long-term successful PTA were enrolled. Their main clinical characteristics at transplantation were: age  $38.6 \pm 9.9$  yrs; males/females 13/22; BMI:  $23.6 \pm 2.8$  kg/m<sup>2</sup>; diabetes duration  $26.1 \pm 10.0$  yrs; fasting plasma glucose (FPG)  $256 \pm 99$  mg/dl; HbA1c:  $8.7 \pm 1.8\%$ ; C peptide:  $0.10 \pm 0.13$  ng/ml. Median post-transplant FU was  $5.0 \pm 1.9$  yrs. All patients were examined before and during the FU by the Sonos 5500 echograph, Agilent Technologies, used by a single operator.

**Results:** All patients had maintained PTA function with sustained normoglycemia without exogenous insulin therapy (FPG  $85 \pm 11$  mg/dl, HbA1c  $5.4 \pm 0.4\%$  and C peptide  $2.91 \pm 1.26$  ng/ml, all  $p < 0.001$  compared to pre-PTA values). The following echocardiographic parameters significantly improved ( $p < 0.05$  or less) at the end of the follow up: left ventricular mass index (LVMI) decreased from  $82 \pm 17$  to  $73 \pm 18$  mg/m<sup>2</sup>, posterior wall thickness during diastole (PWTD) decreased from  $8.1 \pm 1.1$  to  $7.0 \pm 1.0$  mm; interventricular septum thickness during diastole (IVSTD) decreased from  $9.3 \pm 1.4$  to  $8.7 \pm 1.3$  mm, left ventricular ejection fraction increased from  $55.3 \pm 4.3$  to  $57.9 \pm 1.7\%$ ,  $p < 0.001$ . No significant change was observed in a group of matched T1D subjects with failed PTA.

**Conclusion:** Successful pancreas transplant alone in T1D patients was associated with improved cardiac morphology and function in our single centre series.

## OP 26 Causes and consequences of diabetic nephropathy

151

### Gender effects on the modulation of RAGE in a model of type 2 diabetic nephropathy

B.E. Harcourt<sup>1</sup>, A. Morley<sup>2</sup>, K.C. Sourris<sup>2</sup>, M.T. Coughlan<sup>2</sup>, F.Y.T. Yap<sup>2</sup>, S.A. Penfold<sup>2</sup>, J.M. Forbes<sup>1</sup><sup>1</sup>Glycation and Diabetes, Mater Medical Research Institute, Brisbane,<sup>2</sup>Glycation and Diabetes Complications, Baker IDI Heart and Diabetes Institute, Melbourne, Australia.

**Background and aims:** Receptor for advanced glycation end products (RAGE) is a known mediator of the vascular complications of type 2 diabetes including nephropathy. Adipose derived estrogens are able to modulate the expression of RAGE via interaction with transcription factors such as SP-1, AP-1 and NF- $\kappa$ B. The aim of this study was therefore to examine if the presence of more pronounced renal disease in males is due to modulation of RAGE by oestrogen in the diabetic kidney.

**Materials and methods:** We utilised male and female diabetic leptin receptor gene deletion mice (db/db, *Lepr*<sup>+/+</sup>C57BL/KsJ, (n=10) and their appropriate non-diabetic littermate controls (db/H *Lepr*<sup>+/+</sup>C57BL/KsJ, n=10). Mice were followed for 18 weeks and became diabetic at 8–10 weeks. Renal dysfunction, glucose homeostasis, hormone profile and biochemical indices were analysed. The renal RAGE profile was analysed via RT-PCR, ELISA and chromatin immunoprecipitation (ChIP).

**Results:** Renal function was impaired in all db/db mice. Urinary albumin excretion rate (AER) and Cystatin C, were increased in db/db vs. db/H mice, and this was more prominent in male vs. female db/db mice ( $p<0.05$ ). Creatinine clearance also declined in male db/db mice. Glycaemic control, was worse in db/db vs. db/H mice ( $p<0.05$ ), but this was not affected by sex. Renal RAGE expression was significantly increased in male db/db vs. db/H ( $p<0.05$ ), which corresponded with disruptions in oestrone:oestradiol ratios. In male db/db mice, transcriptional regulation within the promoter of RAGE occurred via the novel regulation via transcriptional regulator RAGE itself and via ER- $\alpha$  and ER- $\beta$  complexes. In female db/db mice transcriptional regulator RAGE interacted with NF- $\kappa$ B to exacerbate renal dysfunction.

**Conclusion:** This study demonstrated sex specific differences in the regulation of RAGE which specifically occurred via adipose derived estrogen inducible transcription factors, and resulted in significantly worse renal function in male diabetic mice. Adipose derived hormones and their influence on renal RAGE may account for sex differences commonly seen in the development of diabetic renal disease.

152

### Genetic variability in the RAGE gene and sRAGE levels do not predict progression of diabetic nephropathy and diabetes-related morbidity and mortality

K. Kuricová<sup>1</sup>, V. Tanhäuserová<sup>1</sup>, V. Bartáková<sup>1</sup>, L. Pácal<sup>1</sup>, J. Svojanovský<sup>2</sup>, D. Krusová<sup>2</sup>, J. Olšovský<sup>2</sup>, J. Bělobrádková<sup>2</sup>, J. Řehořová<sup>2</sup>, K. Kaňková<sup>1</sup><sup>1</sup>Dept. of Pathophysiology, Faculty of Medicine, Masaryk university,<sup>2</sup>Dept. of Internal Medicine, St. Anne's Faculty Hospital, <sup>3</sup>Dept. of Gastroenterology, Faculty Hospital Brno-Bohunice, Brno, Czech Republic.

**Background and aims:** Activation of the Receptor for Advanced Glycation Endproducts (RAGE) by its ligands triggers intracellular signal cascade leading to proinflammatory shift of cellular phenotype. Sustained RAGE activation as seen in diabetes causes cellular dysfunction participating in the pathogenesis of micro- and macrovascular diabetic complications. Using cross-sectional association study design we have previously identified RAGE “risk” haplotype (for diabetic nephropathy (DN) comprised of minor alleles of htSNPs -429T/C (rs1800625) and 2184A/G (rs3134940). Endothelial cells produce truncated soluble RAGE (sRAGE) that supposedly acts as natural competitive inhibitor of RAGE signalling, however out previous cross-sectional data identified sRAGE levels as a function of GFR rather than compensatory protective mechanism. Using follow-up data from prospective study of diabetic population of South Moravia region of Czech Republic the aim of our study was to analyse genetic data related to the RAGE gene and sRAGE levels as potential biomarkers of adverse outcomes of diabetes.

**Materials and methods:** Study comprised a total of 412 diabetic subject with variable stage of DN (i.e. normoalbuminuria (n=75), persist. microalbuminuria (n=96), proteinuria (n=147) and ESRD (n=94)) prospectively followed for a median of 39 [IQR 21 - 59] months. Following end-points were considered: [1] progression of DN by stage, [2] major cardiovascular event (MCVE, non-fatal myocardial infarction or stroke, limb amputation) and [3] all-cause mortality (ACM). Genotyping of RAGE variants (-429T/C, -374T/A, G82S, 1704G/T, 2184A/G, 2245G/A) was performed by methods based on PCR. Baseline serum concentration of sRAGE were measured using ELISA (plasma samples were available in 214 subjects). Software PHASE was used to estimate population haplotype frequencies. Kaplan Meier time-to-event analysis was carried out to ascertain contribution of studied parameters to the three endpoints.

**Results:** Comparison of allele and genotype frequencies of RAGE variants between subjects with and without DN revealed differences only in allele and genotype frequencies of 2245G/A polymorphism (chi-square test,  $P=0.0047$  and 0.0148, respectively). Frequencies of the RAGE risk haplotype did not differ significantly ( $P>0.05$ ). Cumulative incidence of progression of DN was 22.9 %, MCVE 8.2 %, and ACM 19.8 %. No significant effects were ascertained for the carrier state of RAGE genotypes/haplotypes and progression of DN, MCVE and ACM (all  $P>0.05$ , log-rank test). For analogous analysis with sRAGE level, subjects were divided into two groups according to the median of sRAGE. We did not identify any significant difference in studied end-points between the groups (all  $P>0.05$ , log-rank test).

**Conclusion:** Our results indicate that neither genetic variation in RAGE gene nor sRAGE level could be used as a robust predictor of diabetes-associated morbidity or mortality.

*Supported by: NT11405 from the Ministry of Health of Czech Republic*

153

### Allelic variations in the superoxide dismutase 2 (SOD2) gene and diabetic nephropathy in subjects with type 1 diabetes

K. Mohammedi<sup>1,2</sup>, S. Maimaitiming<sup>1</sup>, N. Emery<sup>1,3</sup>, N. Bellili-Muñoz<sup>1</sup>, R. Roussel<sup>1,3</sup>, F. Fumeron<sup>1,3</sup>, S. Hadjadj<sup>4,5</sup>, M. Marre<sup>1,3</sup>, G. Velho<sup>1</sup><sup>1</sup>Research Unit 695, INSERM, Paris, <sup>2</sup>Department of Diabetology,Endocrinology and Nutrition, Bichat Hospital, Paris, <sup>3</sup>UFR de Médecine, Univ Paris Diderot, Sorbonne Paris Cité, Paris, <sup>4</sup>Research Unit 927, INSERM, Potiers, <sup>5</sup>UFR de Médecine et Pharmacie, Université de Poitiers, France.

**Background and aims:** Oxidative stress is involved in the pathophysiology of diabetic nephropathy. Manganese superoxide dismutase (Mn-SOD or SOD2) catalyses the dismutation of superoxide, regulates the metabolism of reactive oxygen species in the mitochondria and is highly expressed in the kidney. We investigated associations of SOD2 allelic variations with diabetic nephropathy in type 1 diabetic subjects.

**Materials and methods:** Eight SNPs in the SOD2 region were analysed in 1285 Caucasian type 1 diabetic patients from the SURGENE prospective study (n=340; 10-year follow-up), Genesis France-Belgium (n=501) and GENEDIAB (n=444) cross-sectional studies. The variants give information on ~90% of the allelic variation of SNPs with minor allele frequency  $\geq 5\%$  in haplotype blocks containing SOD2. Incipient nephropathy was defined as persistent microalbuminuria (UAE = 30–300 mg/24 h); established nephropathy as macroalbuminuria (UAE >300 mg/24 h) and plasma creatinine <150  $\mu$ mol/l; and advanced nephropathy as macroalbuminuria and plasma creatinine >150  $\mu$ mol/l or renal replacement therapy. Cox proportional hazards and logistic regression analyses were used to estimate hazard ratios (HR) or odds ratios (OR) for incidence and prevalence of nephropathy. The prevalence of proliferative retinopathy was different in the 3 cohorts (10%, 44% and 82%, respectively) and thus, all analyses were adjusted or stratified by retinopathy stages.  $p\leq 0.01$  was significant.

**Results:** In the SURGENE study, the major T-allele of a functional variant (rs4880, c.T47C, p.V16A) was associated with the prevalence of established/advanced nephropathy at baseline (OR 3.13, 95% CI 1.23 - 8.73,  $p=0.01$ ), with the incidence of microalbuminuria (HR 1.54, 95% CI 1.11 - 2.11,  $p=0.01$ ), with the incidence of renal events (defined as progression to a more severe stage of nephropathy; HR 1.42, 95% CI 1.12 - 1.77,  $p=0.004$ ) and with glomerular filtration rate decline during follow-up (TT:  $-3.96 \pm 0.89$ , CT:  $-1.57 \pm 1.05$ , CC:  $-0.64 \pm 1.12$  ml/min/year,  $p=0.003$ ). Similar results were observed for rs2758329 and rs8031. Pooled cross-sectional analyses were performed in Genesis and GENEDIAB cohorts with stratification by retinopathy stage (non-proliferative/pre-proliferative vs proliferative) and appropriate covariable adjustments to take into account cohort differences. Associations with established/advanced nephropathy were replicated in the subset of subjects



without proliferative retinopathy: OR 4.65, 95% CI 1.64 - 13.82,  $p=0.005$  for rs4880; OR 4.34, 95% CI 1.58 - 12.48,  $p=0.005$  for rs2758329; OR 3.94, 95% CI 1.43 - 11.32,  $p=0.009$  for rs8031. No associations were observed in the subset of subjects with proliferative retinopathy. Results were confirmed in haplotype analyses in all cohorts.

**Conclusion:** SOD2 allelic variations were associated with the prevalence and incidence of diabetic nephropathy and with a faster decline in GFR in type 1 diabetic subjects. These results are consistent with a major role for SOD2 in the protection against oxidative stress and kidney disease in type 1 diabetic patients.

Supported by: AFD, ADRV, AJD

## 154

### Assessment of urinary adiponectin as a predictor of progression for diabetic nephropathy in type 1 diabetes

M. Saraheimo<sup>1,2</sup>, N.M. Panduru<sup>3,4</sup>, C. Forsblom<sup>1,2</sup>, L.M. Thorn<sup>1,2</sup>, J. Wadén<sup>1,2</sup>, A. Bierhaus<sup>5</sup>, P. Humpert<sup>6</sup>, P.-H. Groop<sup>1,2</sup>;

<sup>1</sup>Department of Medicine, Helsinki University Central Hospital, Finland,

<sup>2</sup>Folkhälsan Research Center, Helsinki, Finland, <sup>3</sup>"Carol Davila" University

of Medicine and Pharmacy, Bucharest, Romania, <sup>4</sup>Life Memorial Hospital,

Bucharest, Romania, <sup>5</sup>Department of Medicine I and Clinical Chemistry,

University of Heidelberg, Germany, <sup>6</sup>Stoffwechselzentrum Rhein Pfalz,

Mannheim, Germany.

**Background and aims:** Serum adiponectin has been shown to predict progression of diabetic nephropathy (DN) in type 1 diabetes. Urinary adiponectin was also associated with overt nephropathy in cross-sectional studies in type 2 diabetes, but there are no data regarding the role of urinary adiponectin in the progression of DN in patients with type 1 diabetes. The aim of this study was to assess the predictive value of urinary adiponectin, for the progression at any stage of DN.

**Materials and methods:** This survey is part of the Finnish Diabetic Nephropathy Study. At baseline, out of 2201 patients with type 1 diabetes 1451 had normal AER, 319 microalbuminuria and 320 macroalbuminuria. In addition, 111 non-diabetic subjects were studied. Patients were followed for a median of 5.8 years (95% CI 5.7 - 5.9) during which 101 patients progressed from normo- to microalbuminuria, 42 from micro to macroalbuminuria and 71 from macroalbuminuria to ESRD. Urinary adiponectin levels were measured by ELISA and adjusted with u-creatinine. Different Cox proportional hazard models for the progression at every stage of DN were constructed and used to evaluate the predictive value of urinary adiponectin at any stage of DN.

**Results:** In a cross-sectional analysis, urinary adiponectin significantly increased with worsening nephropathy stage (normo 0.0628 vs micro 0.110 vs macro 0.623  $\mu\text{g}/\mu\text{mol}$ ,  $p<0.0001$ ) and was higher in patients with normal AER than in non-diabetic subjects. In Cox regression analyses, when adjusted for sex and the stage specific progression models, urinary adiponectin predicted progression to a higher stage in the microalbuminuria group (OR=1.37, [95%CI 1.04 - 1.80],  $p=0.025$ ) and also progression to ESRD in the macroalbuminuria group (OR=1.03, [95%CI 1.01 - 1.05],  $p<0.0001$ ). When we compared AER (current gold standard) and urinary adiponectin ROC curves for the prediction of progression to ESRD in the macroalbuminuria group there was no significant difference between the areas under curve ( $\Delta_{\text{AUCs}}=0.00867$ ,  $p=0.17$ ).

**Conclusion:** Urinary adiponectin may serve as a novel biomarker for DN and is as accurate as AER for the prediction of progression to ESRD in patients with macroalbuminuria.

Supported by: Folkhälsan Research Foundation, Stockmann Foundation

## 155

### Copeptin, a surrogate marker for vasopressin, is associated with chronic kidney disease progression in patients with diabetes mellitus (ZODIAC-33)

W.E. Boertien<sup>1</sup>, I.J. Riphagen<sup>1</sup>, I. Drion<sup>2</sup>, A. Alkhalaf<sup>2</sup>, S.J.L. Bakker<sup>1</sup>, K.H. Groenier<sup>3</sup>, J. Struck<sup>4</sup>, H.J.G. Bilo<sup>2,5</sup>, N. Kleefstra<sup>2,5</sup>, R.T. Gansevoort<sup>1</sup>;

<sup>1</sup>Nephrology, UMCG, Groningen, Netherlands, <sup>2</sup>Diabetes Centre, Isala

Clinics, Zwolle, Netherlands, <sup>3</sup>General practice, UMCG, Groningen,

Netherlands, <sup>4</sup>BRAHMS GmbH, Thermo Fisher Scientific, Hennigsdorf,

Germany, <sup>5</sup>Internal Medicine, UMCG, Groningen, Netherlands.

**Background and aims:** Vasopressin is involved in volume homeostasis, but has been hypothesized to have also deleterious renal effects. In diabetic rodent

models vasopressin has indeed been shown to cause glomerular hyperfiltration, albuminuria and glomerulosclerosis. In humans the role of vasopressin in the progression of diabetic nephropathy has yet not been investigated. We studied the association of copeptin, a surrogate for vasopressin, with (change in) albuminuria and eGFR in a cohort of patients with diabetes mellitus, cross-sectionally and during follow-up.

**Materials and methods:** Included were patients with type 2 diabetes, treated in primary care and participating in two prospective cohorts from the ZODIAC study. Plasma copeptin was measured in baseline samples (Chemiluminescence Immunoassay), which were stored at  $-80^{\circ}\text{C}$ . Estimated GFR (MDRD) and urinary albumin/creatinine ratio (ACR) were measured annually. We used multivariate linear regression models to investigate the association between plasma copeptin and ACR increase and eGFR decrease, adjusting for gender, age, baseline ACR or eGFR, chronic kidney disease (CKD) risk factors (being: smoking, HbA1c, blood pressure, total cholesterol and body mass index) and the use of ACE-inhibition.

**Results:** Plasma samples of 1325 diabetes patients were available (44% male, age  $67 \pm 11.6$  yrs, ACR 16 (IQR 8.1-53.6)  $\text{mg/g}$ , eGFR  $65 \pm 15$   $\text{ml/min/1.73m}^2$ ). Baseline plasma copeptin level (5.2 (IQR 3.1 - 9.4)  $\text{pmol/L}$ ) was significantly associated with baseline ACR (standardized B 0.15,  $p<0.001$ ) and eGFR (std B -0.20,  $p<0.001$ ). These associations remained significant after adjustment for gender, age and CKD risk factors (std B 0.12,  $p<0.001$  and std B -0.25,  $p<0.001$  resp.). From this cohort, plasma samples and at least 3 years of follow-up were available in 1184 (89%) patients. These patients were followed for a median of 7.4 (5.9-9.9) years, during which a mean increase in ACR of 0.1  $\text{mg/g/year}$  and a mean decrease in eGFR of 1.2  $\text{ml/min/1.73m}^2/\text{year}$  occurred. Baseline plasma copeptin was significantly associated with rate of ACR increase and eGFR decline after adjustment for gender, age, and baseline ACR or eGFR (std. B 0.10,  $p$  0.02 and std. B -0.08,  $p$  0.01, resp.). These associations remained significant after adjustment for risk factors for CKD progression (std. B 0.09,  $p$  0.04 and std. B -0.07,  $p$  0.03, resp.). These associations were independent of baseline ACR and/or baseline eGFR. In the multivariate regression models the strength of the association of copeptin with either increase in ACR or decrease in eGFR was stronger than that of established risk factors for diabetic nephropathy such as smoking, BMI, blood pressure and HbA1c.

**Conclusion:** In diabetic patients a higher baseline copeptin concentration is significantly associated with an increase in ACR and a decline in eGFR during follow-up, independent of, and stronger than most traditional CKD risk factors. Therefore it would be interesting to study the effect of lowering vasopressin levels (e.g. by drinking more water) on the progression of chronic kidney disease in diabetic patients.

## 156

### Patients with diabetic kidney disease have a high frequency of bacterial infections

M. Lehto, R. Simonsen, V. Harjutsalo, C. Forsblom, P.-H. Groop, the FinnDiane Study Group;

Folkhälsan Research Center, Folkhälsan Institute of Genetics, Helsinki, Finland.

**Background and aims:** Diabetic nephropathy is commonly associated with chronic inflammation. Long disease duration and poor glycemic control increase the risk of bacterial infections in patients with diabetes. Frequent exposure to pathogen associated molecules (e.g. bacterial endotoxins, flagellin, peptidoglycans) may accelerate the development of diabetic complications, especially diabetic nephropathy. The specific aim of the present study is to assess the prevalence of bacterial infections in Finnish patients with type 1 diabetes (T1D) using the data from the national hospital and drug registries.

**Materials and methods:** For the study of bacterial infections, 4023 patients with T1D were selected from the FinnDiane Study. For comparison, we obtained from the Finnish Public Register Center ([www.vaestorekisterikeskus.fi](http://www.vaestorekisterikeskus.fi)) a large control population [10991 non-diabetic control (NDC) subjects], which were age- and sex-matched for the diabetic patients. Information about bacterial infections and antimicrobial drugs used for treatment of infections were collected from a 10-year period (1996-2005) from the Hospital Discharge Register (HILMO; [www.stakes.fi](http://www.stakes.fi)), and the National Drug Register (The Social Insurance Institution of Finland-KELA; [www.kela.fi](http://www.kela.fi)). Total number of follow-up years in non-diabetic controls and different diabetic subgroups were as follows: NDC\_subjects (109271 yrs), T1D\_normal AER (13023 yrs), T1D\_microalbuminuria (2242 yrs), T1D\_macroalbuminuria (3333 yrs), and T1D\_ESRD (2167 yrs). Statistical analyses were performed using SPSS 15.0 and SAS 9.2.

**Results:** T1D patients were diagnosed with various microbial infections 2.5–4.0 times more frequently than the NDC-subjects. Of all microbial infections, bacterial infections were more common in T1D patients compared to NDC-subjects (54 vs. 34%). Based on the total number of follow-up years, the frequency of bacterial infections increases in parallel with the stages of kidney disease [bacterial infections (n)/patient years]: 0.008 NDC-subjects (1.0x), 0.011 T1D patients with normal AER (1.3x), 0.021 T1D patients with microalbuminuria (2.5x), 0.034 T1D patients with macroalbuminuria (4.2x), and 0.237 ESRD (29.0x) [Cochran-Armitage (CA) Trend Test;  $p < 0.0001$ ]. T1D patients purchased antibiotics on average 2.3 times more frequently than NDC-subjects. The number of purchases increased in parallel with the stages of kidney disease [antibiotic purchases (n)/patient years]: 0.46 NDC-subjects (1.0x), 0.79 T1D patients with normal albuminuria (1.7x), 1.02 T1D patients with microalbuminuria (2.2x), 1.33 T1D patients with macroalbuminuria (2.9x), and 3.28 ESRD (7.1x) (CA Trend Test;  $p < 0.0001$ ). In diabetic patients, the number of antimicrobial purchases correlated positively with glycemic control.

**Conclusion:** Bacterial infections are more common in diabetic patients compared to age-matched non-diabetic controls. More importantly, bacterial infections are more frequently encountered in patients with diabetic nephropathy. Whether the bacterial infections are mere consequence or potential contributing factor to diabetic nephropathy has to be elucidated.

*Supported by: Stockmann Foundation, Diabetes Research Foundation, Novo Nordisk Foundation*

## OP 27 New pathways involved in the cross talk between immune cells and metabolic tissues

157

### Role of CD40 and its adaptors protein TRAF in obesity and obesity-associated complications

M. Poggi<sup>1,2</sup>, D. Engel<sup>2</sup>, L. Beckers<sup>2</sup>, E. Wijnands<sup>2</sup>, A. Driessen<sup>2</sup>, M.-C. Alessi<sup>1</sup>, N. Gerdes<sup>3</sup>, E. Lutgens<sup>4</sup>;

<sup>1</sup>Faculty of medicine, INSERM 1062/INRA 1260, Marseille, France, <sup>2</sup>Pathology, Cardiovascular Research Institute Maastricht (CARIM), Netherlands, <sup>3</sup>Poliklinik, Klinikum der Universität München, Ludwig-Maximilians-Universität München, Institut für Prophylaxe und Epidemiologie der Kreislauferkrankungen, Germany, <sup>4</sup>Medical Biochemistry, Academic Medical Center, Amsterdam, Netherlands.

**Background and aims:** Obese adipose tissue shows hallmarks of chronic inflammation, which promotes the development of metabolic disorders and cardiovascular complications such as insulin resistance and type2 diabetes. The mechanisms by which cells interact and the players involved are still unclear. We recently reported that CD40L deficiency prevents adipose tissue inflammation and metabolic manifestations of obesity in mice. Because of serious side-effect of CD40L treatment, our aim is to investigate the role of CD40 receptor and its associated signaling intermediates the TRAFs (TNF-receptor associated factors) in the development of obesity and associated complications.

**Materials and methods:** CD40<sup>+/+</sup>, CD40<sup>-/-</sup> or CD40<sup>-/-</sup> mice bearing a CD40 transgene under an MHCII promoter with or without mutations at the CD40-TRAF2/3/5, CD40-TRAF6 or both binding sites (CD40-TRAF2/3/5/6) were fed a 45% HFD for 18–20 week. Metabolic and inflammatory parameters were evaluated by glucose and insulin tolerance test, flow cytometry, RNA quantification, immunohistochemistry and ELISA.

**Results:** Conversely to CD40L deficiency, CD40-deficiency did not attenuate the development of obesity, but increased CD3<sup>+</sup> infiltration in adipose tissue and glucose intolerance. Absence of CD40-TRAF2/3/5/6 signalling accelerated the body weight gain. Body-weight-matched CD40-TRAF2/3/5/6 mice exhibited a higher insulin resistance and glucose intolerance than other genotypes. In their adipose tissue, pro-inflammatory T cell number was increased while Treg number was reduced. Hepatic steatosis was strongly aggravated in CD40-TRAF2/3/5/6 mice. A similar phenotype but less pronounced was observed when only CD40-TRAF2/3/5 signaling was defective. In contrast, absence of CD40-TRAF6 signalling did not aggravate the HFD-induced effects and even reduced the body weight gain and hyperglycaemia.

**Conclusion:** Unexpectedly, CD40 deficiency aggravates metabolic complications and inflammation associated with obesity. Absence of CD40-TRAF2/3/5 signaling amplifies the deleterious effect of high fat feeding, suggesting a protective role of this pathway. Modulate CD40-TRAF signaling could constitute a therapeutic target to improve obesity and insulin resistance.

158

### Complement C5a anaphylatoxin receptor (C5aR) contributes to macrophage accumulation and polarisation in adipose tissue inflammation

J. Phielers<sup>1</sup>, K.-J. Chung<sup>1</sup>, A. Chatzigeorgiou<sup>1</sup>, J.D. Lambris<sup>2</sup>, T. Chavakis<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine III and Institute of Physiology, Dresden University of Technology, Germany, <sup>2</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, USA.

**Background and aims:** Obesity is associated with chronic low-grade inflammation of adipose tissue (AT) and the development of insulin resistance and type 2 diabetes mellitus. In particular, accumulation of macrophages in the obese AT and their polarization into the M1 pro-inflammatory subtype plays a critical role in the development of insulin resistance. Complement is a versatile system involved in activation of innate immunity, inflammation and tissue remodeling. The central reactions in the complement cascade are the cleavage of C3 and C5 that leave behind the active anaphylatoxins C3a and C5a that act through their cellular G-protein-coupled receptors, C3aR and C5aR, and the opsonins C3b and C5b. Complement components have been implicated as regulators in AT inflammation, however, the exact

influence of the C5a-C5aR axis on the accumulation and activation of pro-inflammatory immune cells within the AT in the course of obesity is poorly characterized.

**Materials and methods:** We performed the high fat diet (HFD)-induced obesity model in mice sufficient or deficient in the C5aR. Therefore, 8–9 weeks old mice were fed a HFD or control normal diet (ND) for 20 weeks. Insulin resistance development was tested by i.p. glucose tolerance test (GTT) and i.p. insulin tolerance test (ITT) (week 16 or 17 of the feeding, respectively). Subcutaneous and peri-gonadal fat pads were excised, digested with collagenase and the stromal vascular fraction (SVF) was analyzed by fluorescence activated cell sorting (FACS). In addition, excised adipose tissues are currently analyzed by immunohistology and Western Blot.

**Results:** We found, that expression of C5aR was increased 10 times in subcutaneous and 100 times in gonadal AT of obese mice compared to lean mice. Moreover, the total weight gain upon HFD was not affected by C5aR-deficiency, however, obese mice lacking C5aR displayed improved glucose tolerance. This was associated with reduced AT inflammation due to C5aR deficiency. In particular, while the number of total leukocytes and infiltrated CD4<sup>+</sup> or CD8<sup>+</sup> T-cells did not differ between C5aR-sufficient and -deficient mice, we observed that the numbers of total macrophages and especially the proinflammatory M1 type (defined as F4/80+CD11b+CD11c<sup>+</sup> and / or F4/80+CD11c+CD206<sup>+</sup> cells) were significantly reduced ( $p < 0.05$ ) in C5aR<sup>-/-</sup> mice, as compared to WT controls. In addition, we could detect increased expression of the M2-derived anti-inflammatory cytokine IL-10 and reduced expression of proinflammatory cytokines such as TNF and IL-6 in C5aR-deficient animals ( $p < 0.05$ ).

**Conclusion:** These results suggest that the C5a-C5aR axis contributes to macrophage accumulation and polarization into M1 cells in the obese AT and thereby to insulin resistance development.

## 159

### Myeloid specific knockout of ADAM17 protects from diet induced insulin resistance and hepatic inflammation

V. Casagrande, A. Marino, M. Fabrizi, R. Menghini, R. Lauro, M. Federici; University of Tor Vergata, Medical School, Rome, Italy.

**Background and aims:** Tissue Inhibitor of Metalloprotease 3 (TIMP3), an endogenous regulator of metalloproteinases such as MMP9 and TNF- $\alpha$  Converting Enzyme (TACE, also named ADAM17), acts at the interface of inflammation and insulin resistance. Timp3 null mice fed a high fat diet (HFD) showed glucose intolerance, insulin resistance and macrovesicular steatosis compared with WT mice. Moreover, obesity is characterized by a deficiency of TIMP3 in liver and white adipose tissue (WAT), suggesting that downregulation of TIMP3 in metabolic tissue may contribute to the deregulation of inflammatory pathways. Reduced Timp3 expression results in uncontrolled ADAM17 protease activity increasing the level of circulating soluble TNF- $\alpha$ . On the other hand ADAM17 deficiency confers partial protection from HFD-induced insulin resistance, with positive effects on liver ability to resist to inflammatory effects of HFD. Here we test the effect of selective inhibition of ADAM17 in hepatocyte or myeloid cells on glucose metabolism and inflammatory status.

**Materials and methods:** To generate knockout animals we used the Cre/loxP strategy. We crossed mice expressing Cre recombinase under control of Albumin or Lysozyme2 promoters with mice that containing loxP sites flanking ADAM17 gene. ADAM17 hepatocytes (A17LKO) and myeloid cells (A17M-KO) knockout mice were confirmed by PCR analyses. WT (Adam17 Floxed), A17LKO and A17MKO mice fed standard chow or HFD were subjected to metabolic and molecular phenotypization.

**Results:** In the diet induced obesity model, after 16 weeks of HFD, we observed that A17LKO mice did not differ in weight from WT mice while A17MKO showed a reduced weight. Consistently, A17MKO mice compared with WT and A17LKO, showed a significant improved fasting glucose levels, glucose tolerance and insulin sensitivity, measured by intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (IPITT). We analyzed ADAM17 expression in liver by Real-Time PCR and Western blot, and showed that ADAM17 levels were significantly decreased in both transgenic mouse models. Further analysis on isolated hepatic cells showed that ADAM17 mRNA was significantly reduced in hepatocytes but not in non-parenchymal cells from A17LKO mouse livers. Moreover, there was a reduction of ADAM17 mRNA levels in CD11b<sup>+</sup> isolated cells from A17MKO mouse liver. Western blot analysis on liver extracts revealed that JNK1/2 were less phosphorylated in A17MKO compared to A17LKO and controls. In liver mRNA expression profiling for lipogenic and inflammatory genes revealed

that A17MKO mice had significant lower levels for TNF- $\alpha$ , CCL2 and CD68, ChREBP and SCD1.

**Conclusion:** Our data indicate that the myeloid specific ADAM17 deletion protects from HFD induced insulin resistance and hepatic inflammation.

Supported by: FP7-Health-241913 FLORINASH

## 160

### TimP3 modulates immune cell activation in a model of metabolic inflammation

V. Marchetti, M. Fabrizi, M. Cavallera, M. Mavilio, V. Casagrande, R. Menghini, R. Lauro, M. Federici; Internal Medicine, University of Rome Tor Vergata, Italy.

**Background and aims:** Activation of inflammatory pathways contributes to the beginning and the development of both atherosclerosis and type-2 diabetes. Among other cytokines, tumour necrosis factor (TNF) is central to the initiation and orchestration of innate immunity and recruitment of circulating leukocytes and dendritic cells to inflamed sites. TNF- $\alpha$  converting enzyme (TACE) mediates TNF shedding and is selectively inhibited by the tissue inhibitor of metalloproteinase 3 (TIMP3). Decreased expression of TIMP3 results in insulin resistance and inflammation while a TACE inhibitor improves insulin sensitivity and vascular inflammation. Here, we investigate whether TIMP3 affects inflammatory cells phenotype elucidating their function during the progression of metabolic dysfunction.

**Materials and methods:** We evaluated the effects of TIMP3 deficiency on systemic and tissue leukocytes and their state of activation in mice, under conditions of high fat diet (HFD) (1–4 months). Leukocytes were isolated from blood and stromal vascular cell (SVC) fraction of white adipose tissue (WAT) and characterized by flow cytometry antibodies direct against CD11c, CX3CR1, F4/80, and CD206 antigens. Real Time PCRs were engaged in order to evaluate and quantify the expression of pro and anti-inflammatory genes in SVC of WT and TIMP3KO mice after the HFD treatment. The level of circulating pro and anti-inflammatory mediators was studied using a panel of 60 cytokines.

**Results:** The total number of circulating F4/80<sup>+</sup>, F4/80<sup>+</sup> CX3CR1<sup>+</sup>, CX3CR1<sup>+</sup>, CX3CR1<sup>+</sup>CD11c<sup>+</sup> was found increased in TIMP3KO mice compared to the WT animals at 3 months of HFD treatment (1.9, 3.8, 2.3, 2.9 fold increase respectively,  $p < 0.001$ ), while CD11c<sup>+</sup>CD206<sup>+</sup> cells were found decreased in KO animals compared to WT mice (1.8 fold change,  $p < 0.001$ ). TIMP3KO, compared to WT, revealed early signs of glucose intolerance after 2/3 months of HFD and were overtly hyperglycaemic at glucose tolerance test, independently from body weight ( $p < 0.01$ ), at 4 months of HFD. F4/80<sup>+</sup>CX3CR1<sup>+</sup>, CX3CR1<sup>+</sup>CD11c<sup>+</sup>, CX3CR1<sup>+</sup>CD11c<sup>-</sup> cells were significantly increased in the SVC of TIMP3 KO mice compared to WT under HFD for 4 months. Several proinflammatory genes, distinctive of macrophage M1 and dendritic cells were significantly up regulated in the SVC of TIMP3 KO mice compared to the WT mice (MGL1, IFN- $\gamma$ , IL1 $\beta$ , CX3CR1 and CX3CL1), while genes of M2 macrophage line were undetectable in the SVC of TIMP3 mice compared to SVC of WT animals (IL-10, YM1). The level of proinflammatory cytokines was significantly upregulated in the serum of TIMP3 KO mice compared to the serum of WT mice (IL-6, MIP1- $\alpha$  and - $\gamma$ , IL-8).

**Conclusion:** We have identified one population of F4/80<sup>+</sup>CX3CR1<sup>+</sup> macrophages and CD11c<sup>+</sup>CX3CR1<sup>+</sup> dendritic cells (DCs) that are upregulated along the HFD condition in the blood and SVC component of TIMP3KO mice. How these myeloid cells participate in inflammatory signals in adipose tissue is not completely known. Because myeloid cells accumulation and resident macrophage polarization is a critical event regulated by TIMP3 for the coordination of inflammatory events within fat tissue, increasing its release from cells directly at tissue injury site may be a valid approach for decreasing inflammatory signals in adipose tissue.

Supported by: Telethon GGP08065, Fondazione Roma

## 161

### The phenotype of infiltrating macrophages influences arteriosclerotic plaque vulnerability in the carotid artery

K. Cho, T. Kondo, T. Atsumi, H. Miyoshi; Department of Medicine II, Hokkaido University Graduate School of Medicine, Sapporo, Japan.

**Background and aims:** Macrophages located in the wall of the artery accumulate excess lipid, become foam cells and contribute to atherosclerotic



plaque development. Continued accumulation of lipid by foam cells is associated with an inflammatory response and increased plaque vulnerability. Vulnerable plaques are less stable and associated with major cardiovascular events. Obesity is known to cause a reduction in M2 macrophages (anti-inflammatory) in adipose tissue and an increase in the numbers of M1 macrophages (inflammatory) which have been likened to foam cells. However, there are limited studies investigating the relationship between macrophage polarity (M1 vs M2) and the vulnerability of human atherosclerotic plaques. Therefore, we examined the nature of the macrophage and factors associated with arteriosclerosis using the plaques obtained in carotid endarterectomy (CEA) for stenosis of internal carotid artery.

**Materials and methods:** This study was approved by the ethics and clinical research committee. Patients were provided an overview of the study procedures and informed consent was obtained from eligible patients prior to enrollment in the study. Patient eligibility was determined using criteria described by the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and Japanese Guidelines for the Management of Stroke. An Echocardiogram of the carotid artery was performed in all patients prior to surgery. Plaques were removed from 65 patients via CEA and immediately processed for later analysis by immunohistochemistry, western blotting, and real time-PCR. Patient medical records were obtained from the hospital for additional information regarding medical history and recent blood chemistry data to see related risk factors for atherosclerosis.

**Results:** Patients were divided into two groups, those suffering from cerebral infarction (symptomatic,  $n=35$ ) and those that did not present with symptoms (asymptomatic,  $n=30$ ). Age, sex, BMI, medical history, medication status, blood chemistry, and carotid stenosis were not significantly different between groups. Echocardiogram analysis revealed that plaque vulnerability was greater in the symptomatic group ( $p=0.033$ ,  $\chi^2$ -square test). Analysis by immunohistochemistry demonstrated that plaques from the symptomatic group had a greater concentration of M1 macrophages (CD68, CD11c positive) while plaques from the asymptomatic group had more M2 macrophages (CD163 positive). This observation was confirmed by Western blotting. Further characterized was performed by real time-PCR and we observed that plaques from the symptomatic group had increased expression of the M1 markers CD68 (3.7-fold) and CD11c (3.3-fold), as well as MCP-1 (3.4-fold), IL-6 (9.6-fold), and MMP-9 (10.6-fold) as compared to the asymptomatic group.

**Conclusion:** Here we report that the M1/M2 macrophage activation previously described in adipose tissue was also observed in human arteriosclerotic plaques of the carotid artery. Furthermore our data suggest that macrophage polarity/activation is associated with plaque vulnerability.

anti-CXCL10 treated mice. CXCL10 positive  $\beta$ -cells were found in HFD but not in ND or anti-CXCL10/HFD treated mice. In line with this, mRNA levels of IL-1 $\beta$  and CXCL10 in isolated islets were highly up-regulated (15-fold,  $p<0.05$ ) in HFD treated mice, which was inhibited in anti-CXCL10 treated mice. CXCL10 mRNA was 5-fold up-regulated in epididymal fat and normalized by anti-CXCL10. It was also upregulated in the liver, but in contrast, in the hypothalamus, neither CXCL10 nor IL-1 $\beta$  was changed in HFD or anti-CXCL10 treated mice. The protective effects of anti-CXCL10 were also confirmed in human islets. The cytokine mixture IL-1 $\beta$ /IFN- $\gamma$  highly induced IL-1 $\beta$  production and secretion of IL-1 $\beta$ , which was inhibited by anti-CXCL10, possibly through a negative feedback mechanism. In parallel, cytokine-induced apoptosis was also inhibited.

**Conclusion:** Our results show that CXCL10 antagonism improved  $\beta$ -cell survival and function and support a potential role for CXCL10 in the treatment of diabetes.

*Supported by: DFG*

## 162

### CXCL10 antagonism improves insulin sensitivity, inflammation, beta cell function, survival and mass

P. Shah<sup>1</sup>, E. Domsgen<sup>1</sup>, J. Bergemann<sup>1</sup>, P. Janssen<sup>1</sup>, N. Stappenbeck<sup>1</sup>, P. Cardarelli<sup>2</sup>, K. Maedler<sup>1</sup>;

<sup>1</sup>Centre for Biomolecular Interactions, University of Bremen, Germany,

<sup>2</sup>Medarex Inc., Bristol-Myers Squibb R&D, Sunnysvale, USA.

**Background and aims:** Sub-clinical inflammation participates in the pathophysiology of type 2 diabetes (T2DM) and leads to  $\beta$ -cell failure and impaired function. The pro-inflammatory chemokine CXCL10 is expressed in islets in T1DM and T2DM and leads to  $\beta$ -cell apoptosis and impaired function mediated through TLR4 signaling. In the present study we investigated whether CXCL10 antagonism improves glycemia,  $\beta$ -cell function and survival in vivo in mice and in vitro in human islets.

**Materials and methods:** C57BL/6J mice were fed a high fat/high sucrose diet (HFD) for 16 weeks and treated with an antibody to CXCL10 or with control IgG twice weekly. Glucose and insulin tolerance were monitored every 4 weeks, pancreas, liver, adipose tissue and hypothalamus were isolated and analyzed by IHC and RT-PCR after 16 weeks.

**Results:** A murine CXCL10 antibody prevented diabetes progression in C57BL/6J mice fed a high fat/high sucrose diet (HFD) for 16 weeks. Anti-CXCL10 improved glucose tolerance, insulin sensitivity and glucose stimulated insulin secretion. HFD feeding reduced stimulatory index by 2.8-fold, but anti-CXCL10 treatment completely restored GSIS. While anti-CXCL10 treated mice showed a 1.8-fold increased  $\beta$ -cell mass compared to normal diet (ND) controls,  $\beta$ -cells were unable to compensate for the higher insulin demand in response to the HFD in vehicle treated mice. Analysis of  $\beta$ -cell apoptosis showed an induction of TUNEL-positive  $\beta$ -cells in the HFD mice compared to ND mice (0.2% vs. 0.05%,  $p<0.05$ ), which did not occur in the

## OP 28 Type 1 diabetes mellitus: acute and chronic complications

163

**Insulin resistance is associated with macroangiopathy in type 1 diabetic patients treated with intensive insulin therapy from the onset of the disease**

A. Araszkiwicz, A. Uruska, S. Pilacinski, D. Naskret, B. Wierusz-Wysocka, D. Zozulinska-Ziolkiewicz;  
Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poland.

**Background and aims:** The aim of the study was to evaluate the relationship between insulin resistance and the early marker of macroangiopathy such as intima-media thickness (IMT) in patients with type 1 diabetes, treated from the initial diagnosis with intensive insulin therapy.

**Materials and methods:** The study group consisted of 79 patients with type 1 diabetes (51 men, 28 women) from the Poznan Prospective Study (PoProStu), aged  $37.2 \pm 6.2$  years, with mean HbA1c from 13 years of follow  $8.3 \pm 1.3\%$ . IMT of the right common carotid artery was determined using high resolution ultrasonography (Accuscan Cv 70, Siemens, Erlangen, Germany) with 10-MHz transducer. Two longitudinal projections were assessed (anterolateral and posterolateral). Images were captured at 16 frames per second for 5 s. The distal 1 cm of the common carotid artery just proximal to the bulb was measured and calculated automatically with the Carotid Analyzer for Research (CAD 5) program. Insulin resistance was assessed on the basis of anthropometric data and estimated glucose disposal rate (eGDR) with cut-off point  $7.0 \text{ mg/kg/min}$ . Patients with  $\text{eGDR} < 7.0 \text{ mg/kg/min}$  were defined as insulin resistant.

**Results:** Patients with  $\text{eGDR} < 7.0 \text{ mg/kg/min}$  ( $n=27$ , 36%) had significantly higher IMT [ $0.63(0.55-0.72)$  vs  $0.55(0.50-0.58) \text{ mm}$ ,  $p=0.004$ ]. A significant positive correlation with IMT was revealed for body mass ( $r=0.26$ ,  $p=0.025$ ), BMI ( $r=0.28$ ,  $p=0.015$ ), waist circumference ( $r=0.34$ ,  $p=0.003$ ), WHR ( $r=0.24$ ,  $p=0.041$ ) and negative correlation was observed for  $\text{eGDR}$  ( $r=-0.44$ ,  $p<0.0001$ ). In multiple linear regression model low  $\text{eGDR}$  was associated with increased IMT ( $\beta -0.40$ ,  $p=0.003$ ). The association was independent from duration of diabetes, sex and LDL (low density lipoproteins) concentration.

**Conclusion:** The lower insulin sensitivity the higher IMT in patients with type 1 diabetes, treated from the initial diagnosis with intensive insulin therapy.

Clinical Trial Registration Number: NCT01411033

Supported by: Polish Ministry of Science and Higher Education NN402357238

164

**Early detection of atherosclerosis in asymptomatic patients with type 1 diabetes (concordance between single-photon emission computed tomography, calcium score and carotid ecography)**

E. Aguilera<sup>1</sup>, M. Milà<sup>2</sup>, S. Serrano<sup>3</sup>, E. Bernal<sup>3</sup>, E. Pizarro<sup>4</sup>, I. Olaizola<sup>1</sup>, E. Serra<sup>1</sup>, E. Guanyabens<sup>1</sup>, M. Puig-Domingo<sup>1</sup>;

<sup>1</sup>Endocrinology and Diabetes Unit, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, <sup>2</sup>Nuclear Medicine Unit, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, <sup>3</sup>Cardiology Unit, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, <sup>4</sup>Endocrinology and Diabetes Unit, Hospital de Mataró, Spain.

Type 1 diabetes mellitus (T1D) is associated with an increase in cardiovascular disease, although there are few data on the prevention and screening of these patients. Different studies have demonstrated the functional and structural vascular changes produced in early stages of the evolution of T1D. Carotid artery intima-media thickness (cIMT) is a marker of atherosclerosis which is correlated with coronary disease. Multiple slice computerized tomography (CT) allows anatomical images of the coronary arteries to be non-invasively obtained by the quantification of calcification (calcium score). Myocardial perfusion imaging with single-photon emission computed tomography (SPECT) is commonly used to identify patients with coronary artery disease.

**Aim:** Evaluate the presence of early atherosclerosis in asymptomatic T1D patients with an evolution of more than 10 years and no history of ischemic or macrovascular heart disease, compared with non-diabetic age and sex matched controls.

**Material and methods:** 109 T1D patients consecutively recruited from the outpatient clinic were studied (61 males; age:  $38.2 \pm 8.2$  years,  $21.2 \pm 8.3$  years of T1D evolution; BMI:  $25.1 \pm 3.6 \text{ kg/m}^2$ , HbA1c:  $7.9 \pm 1.1\%$ ; total cholesterol  $182.8 \pm 23.9 \text{ mg/dl}$ ; HDL  $60.4 \pm 15.2 \text{ mg/dl}$ , LDL  $105.9 \pm 21.6 \text{ mg/dl}$ ; 52% non-smokers; 28% retinopathy; 11% microalbuminuria) and 44 non-diabetic controls age and sex matched were studied. Carotid ecography was performed to determine the mean cIMT (common carotid, bifurcation and right and left internal) and the presence of atheroma plaques. A high resolution multidetector CT with ECG was undertaken for calcium analysis and quantification. In T1D patients an SPECT myocardial was also performed.

**Results:** In T1D patients the mean cIMT was significantly greater compared with controls ( $0.56 \pm 0.14$  vs.  $0.48 \pm 0.14 \text{ mm}$   $p=0.004$ ). Patients with atheroma plaques (13/109) presented a significantly higher HbA1c ( $8.7 \pm 0.9\%$  vs.  $7.8 \pm 1.1\%$   $p=0.003$ ) and a greater mean cIMT than patients without plaques ( $0.53 \pm 0.11 \text{ mm}$  vs.  $0.75 \pm 0.14 \text{ mm}$   $p=0.001$ ). Patients with a calcium score  $> 0$  (22/109) displayed a significantly higher age and HbA1c ( $44.5 \pm 5.1$  vs.  $36.7 \pm 8.1$  years  $p=0.001$  and  $8.5 \pm 1.1$  vs.  $7.8 \pm 1.0\%$   $p=0.003$ ), a longer evolution of the disease ( $25.4 \pm 9.2$  vs.  $19.3 \pm 7.4$  years  $p=0.005$ ) and a greater mean cIMT ( $0.53 \pm 0.11 \text{ mm}$  vs.  $0.67 \pm 0.18 \text{ mm}$ ,  $p=0.001$ ) compared to patients with score of 0. In the control group the percentage of subjects with plaques (3/44: 6.8%) and score  $> 0$  (5/44: 11.3%) was lower than in T1D patients. 13/109 T1D patients showed an abnormal SPECT (6 patients presented ischemia and 7 a pathological decline in cardiac ejection fraction after exercise). However, there was a low concordance between calcium score, carotid ecography and SPECT (only 2 patients presented plaques and an score  $> 0$ ).

**Conclusion:** A considerable percentage of our T1D patients with more than 10 years of disease evolution presented data suggestive of atherosclerosis, thus the inclusion of non-invasive screening methods for the detection of early atherosclerosis should be considered in routine clinical practice.

Supported by: Ajut a la Recerca en Diabetis Gonçal Lloveras 2010

165

**The significant influence of integration of glycaemic control on skin AF value through retrospective and prospective studies in patients with type 1 diabetes**

S. Hoshina, J. Miura, E. Sugisawa, H. Kobayashi, M. Osawa, Y. Uchigata;  
Diabetes center, Tokyo Women's Medical University, Japan.

**Background and aims:** It is known that Advanced Glycation Endproducts (AGEs) participates in the development and the progression of the diabetic microvascular complications. Recently Autofluorescence (AF) reader (AGE Reader<sup>®</sup>; DiagnOptics Inc.) which could measure skin AF value in the skin non-invasively was developed. We have reported that skin AF values positively correlated with the progression of the diabetic complications. However, it is not clear what term of glycemic control influenced skin AF value and if change of glycemic control influenced skin AF value in the future. Therefore, we retrospectively investigated the correlation between skin AF values and glycemic control in the past 15 years, and prospectively studied that the relationship between the change of glycemic control and later skin AF value.

**Materials and methods:** We recruited 241 type 1 diabetes patient (Male/Female: 132/109) that regularly visit our hospital, aged  $37 \pm 10$  years old, duration of diabetes  $18.1 \pm 10$  years. Skin AF value was measured on inner side of right forearm on AGE Reader<sup>®</sup>. We measured blood glucose, HbA1c, lipid and blood pressure on the same time. In the retrospective study, we calculated the area under the curve (AUC) of HbA1c every 3 months for 15 years. In prospective study, we could followed 73 patients on skin AF value every 6 months for 2 years. The change of skin AF was defined that increasing 0.1 or more was increasing skin AF group and decreasing 0.1 or more was decreasing group and change less than 0.1 was invariant group.

**Results:** Skin AF value at the enrollment positively correlated with current age, duration of diabetes, HbA1c, LDLc and log ACR ( $R=0.28$ ,  $p=0.002$ ;  $R=0.42$ ,  $p<0.0001$ ;  $R=0.52$ ,  $p<0.0001$ ;  $R=0.22$ ,  $p=0.036$ ;  $R=0.27$ ,  $p=0.01$ , respectively). In retrospective study, the skin AF was strongly correlated with the past HbA1cAUC going back to 15 years (5 years,  $R=0.35$ ,  $p<0.0001$ ; 10 years,  $R=0.36$ ,  $p<0.0001$ ; 15 years,  $R=0.55$ ,  $p<0.0001$ ). In the prospective study, 25/74 patients belonged to increasing, invariant and decreasing groups after 2 years, respectively. Skin AF value was  $2.6 \pm 0.6$  in increase group and  $2.2 \pm 0.6$  in decrease group after 2 year. Skin AF values at 2 years later were not correlated with HbA1c, lipid profile at the time of enrollment and duration of diabetes. Skin AF values at 2 years later were not correlated with HbA1cAUC during 6, 12, 18 and 24 months after registration in all patients (6 months  $R=0.182$ ,  $p=0.124$ ; 12 months  $R=0.169$ ,  $p=0.153$ ; 18 months  $R=0.160$ ,  $p=0.175$ ; 24 months  $R=0.171$ ,  $p=0.149$ ), however, weakly correlated with

HbA1cAUC during 6, 12, 18 and 24 months in decreasing group (6 months  $R=0.37$ ,  $p=0.017$ ; 12 months  $R=0.35$ ,  $p=0.023$ ; 18 months  $R=0.33$ ,  $p=0.035$ ; 24 months  $R=0.33$ ,  $p=0.036$ ). In multivariate regression analysis, skin AF value at 2 years later was most correlated with duration of diabetes duration ( $\beta=0.023$ ,  $p=0.000$ ). Concerning diabetic microvascular complications, 9 patients developed one stage of retinopathy and 5 patients developed one stage of nephropathy. There were no significant difference in skin AF value at the enrollment and change of skin AF value for 2 years between progression group and other group both in retinopathy and nephropathy.

**Conclusion:** The skin AF value was strongly correlated with past HbA1cAUC for more than 5 years. In prospective study, HbA1cAUC for 2 years did not strongly influence on later skin AF value and longer-term observation will be needed.

## 166

### Influence of age at diagnosis over the glycaemic control evolution in type 1 diabetes

L. Forga<sup>1</sup>, M.J. Goñi<sup>1</sup>, D. Mozas<sup>1</sup>, B. Ibañez<sup>2</sup>, K.I. Cambra<sup>2</sup>, J.P. Martínez<sup>1</sup>;

<sup>1</sup>Navarra Hospital Complex, <sup>2</sup>Centro de Investigación Biomédica de Navarra, Pamplona, Spain.

**Background and aims:** The most important factor in order to reach a good metabolic control in type 1 diabetes is a tailored insulin treatment, but some other factors can influence the glycaemic control long after the diagnosis. One of these factors is the age of onset. In patients <20 years of age, the oldest, especially during adolescence have poor glycaemic control. In adults, there are no studies dealing with the relationship between the age of diagnosis and subsequent metabolic control. The aim of this study is to know the effect of age of diagnosis over glycaemic control measured as HbA1c along of follow-up.

**Materials and methods:** This is an observational retrospective follow-up study. The subjects of the study are all patients with type 1 diabetes with onset from 1990 to 2008 that have been treated in our hospital. Patients with LADA were excluded. Information about their HbA1c levels and other clinical parameters were gathered at onset and along the follow-up (until 2010) at a yearly basis. To assess the evolution of HbA1c since the disease onset and the relationship between age at onset and calendar year, we used General Additive Models (GAM) and linear models, with patients' annual HbA1c levels as response variable, and the number of years since the onset together with the age at onset as covariates. This study was approved by the regional Ethics Review Board of our Community.

**Results:** Mean (SD) follow-up was 10.1 (5.3) years, and ranged from 2 (those with onset in 2008) to 20 years (those with onset in 1990). The total number of participants has been 716, 280 with onset under 15 yrs of age and 436 with onset above this age. The proportion of men was slightly higher than the women. Children diagnosed at ≤4 years of age, at the beginning had higher HbA1c levels than the other children under 15. From the third year since onset and afterwards, the evolution of children 0–4 and 5–9 was very similar. Patients with onset between 10 and 14 had a different graph, their HbA1c level at the beginning was similar to the patients aged 5 to 9, but then it rose to reach 8.4% after seven years and then made a plateau. Their final HbA1c levels were lower than the other children groups. In adults, patients diagnosed between 15 and 29 had a rapid increase in the HbA1c level during the four first years and then they made a plateau at 8%. The group with diagnosis between 30 and 44 years had the lowest level of HbA1c at onset, but then it increased slowly and reached 8% level eight years after the diagnosis. Then, the glycaemic control impairment followed. Finally, patients diagnosed between 45 and 60 years had the worst HbA1c levels all the time, from the beginning to the end of the study.

**Conclusion:** 1) The age of diagnosis is related to the glycaemic control evolution in type 1 diabetes 2) Patients aged 10–14 have different behavior than the two other children groups. 3) In adults, patients aged 15–29 and 30–44 show a contrary evolution in their metabolic control during follow-up and 4) patients over 45 have the poorest glycaemic control.

Supported by: ISCIII (PI10/02715) and SNS (53/2008)

## 167

### Subcortical volume loss in type 1 diabetes patients relates to cognitive dysfunction

E. van Duinkerken<sup>1</sup>, R.G. IJzerman<sup>1</sup>, M.M. Schoonheim<sup>2</sup>, M. Klein<sup>3</sup>, F.J. Snoek<sup>3</sup>, F. Barkhof<sup>2</sup>, M. Diamant<sup>1</sup>;

<sup>1</sup>Diabetes Center / Department of Internal Medicine, <sup>2</sup>Department of Radiology, <sup>3</sup>Department of Medical Psychology, VU University Medical Centre, Amsterdam, Netherlands.

**Background and aims:** Type 1 diabetes mellitus (T1DM) is associated with cognitive dysfunction, most notably in patients with microangiopathy. Decrements in performance are particularly seen in domains of processing and motor speed, and attention. These cognitive defects are only weakly associated with global and regional cortical grey matter volume changes in T1DM patients. Hitherto, no studies have assessed the effect of T1DM and microangiopathy on deep grey matter structures and their relation with cognition. Therefore, we assessed subcortical structures in patients with and without microangiopathy and controls and determined the relationship with cognitive dysfunction.

**Materials and methods:** Fifty patients with, 53 without microangiopathy and 47 controls were included in this study. All underwent a multi-sequence MRI-scan on a 1.5T Siemens Sonata (Erlangen, Germany) and a detailed neuropsychological assessment covering general cognitive ability, memory, information processing speed, executive, attentional, motor and psychomotor performance. Using a T1-based magnetization prepared rapid acquisition gradient echo (MPRAGE), subcortical structures were segmented by using FMRIB's Integrated Registration and Segmentation Tool (FSL4.1.5 FIRST). Bilaterally, the nucleus accumbens and caudatus, amygdala, hippocampus, pallidum, putamen, thalamus, and the brainstem were segmented. Mean volume per participant was normalised for head size, to allow group comparison.

**Results:** Patients with microangiopathy were oldest, had higher HbA1c, systolic blood pressure, depressive symptoms and performed worse on cognitive tests involving general cognitive ability, information processing and motor speed, and attention. To reduce statistical tests, all deep grey matter structures were entered in 1 MANCOVA, corrected for age, gender, systolic blood pressure, depressive symptoms and HbA1c. The overall F-test was significant ( $F=1.61$ ;  $P=0.027$ ) allowing post-hoc testing. After Bonferroni correction, this revealed volume loss in the bilateral nucleus accumbens, putamen and thalamus in patients with microangiopathy versus controls (all  $P<0.04$ ). Lower volume in those without microangiopathy relative to controls was borderline significant for the left nucleus accumbens and thalamus, and bilateral putamen (all  $P<0.08$ ). In all patients higher volume of the above mentioned structures was positively related with general cognitive ability, information processing and motor speed ( $r_{\text{max}}=0.3$ ; all  $P<0.04$ ).

**Conclusion:** We found lower volume in the bilateral nucleus accumbens, putamen and thalamus in patients with microangiopathy, which were marginally significant in those without microangiopathy. As these structures, and especially the thalami, have many connections with other (sub)cortical structures, involvement in speed tasks is not surprising, and may indicate that these deep grey matter structures play an important role in T1DM related cognitive dysfunction. Further longitudinal studies should determine the course of these changes, of their interrelationship and the clinical impact. Supported by: Dutch Diabetes Research Foundation Grant 2005.00.069 and EFSD/Lilly grant

## 168

### Increased mortality in autoimmune diabetes in adults

L. Olsson<sup>1</sup>, A. Ahlbom<sup>1</sup>, T. Andersson<sup>1</sup>, V. Grill<sup>2</sup>, K. Midtthjell<sup>3</sup>, S. Carlsson<sup>1</sup>;

<sup>1</sup>Department of epidemiology, Karolinska Institutet, Stockholm, Sweden,

<sup>2</sup>Department of Cancer Research and Molecular Medicine, the Norwegian University of Science and Technology, Trondheim, <sup>3</sup>Department of Community Medicine and General Practice, the Norwegian University of Science and Technology, Levanger, Norway.

**Background and aims:** Knowledge on mortality in autoimmune diabetes with adult onset is limited. Our aim was to analyse the risk of all-cause mortality, and mortality from cardiovascular disease (CVD) and ischemic heart disease (IHD) in autoimmune diabetes in adults compared to individuals without diabetes and individuals with type 2 diabetes.

**Materials and methods:** We used information from 61,613 individuals without diabetes, 208 cases of autoimmune diabetes in adults (anti-GAD positive and diagnosed at age ≥35 years) and 2,425 cases of type 2 diabetes (anti-GAD



negative and diagnosed at age  $\geq 35$  years) who in 1995–1997 participated in the Norwegian Nord-Trøndelag Health Study (consisting of questionnaires and clinical examinations) and who were followed in the Norwegian national cause of death registry until 2009. Hazard ratios (HR) with 95% CI for all-cause, CVD and IHD mortality were calculated using Cox proportional hazards models.

**Results:** Autoimmune diabetes in adults was associated with an increased risk of all-cause (HR 1.56, 95% CI 1.26–1.93), CVD (1.89, 1.42–2.52) and IHD mortality (HR 2.44, 1.61–3.73), similar to the increased mortality risks observed in type 2 diabetes; HR 1.43 (95% CI 1.33–1.53) for all-causes, HR 1.73 (1.58–1.90) for CVD and HR 2.17 (1.91–2.47) for IHD. The excess mortality was seen in both men and women and was not explained by overweight, lifestyle or socioeconomic position. In autoimmune diabetes, mortality was primarily observed in cases with HbA1C  $\geq 6.5\%$ ; HR 2.79 (95% CI 1.78–4.39) vs. HR 1.33 (95% CI 0.33–5.33) for IHD mortality.

**Conclusion:** Autoimmune diabetes in adults is associated with increased mortality risk, primarily from CVD, of similar magnitude as type 2 diabetes. The increased risk is associated with glycaemic control, rather than with traditional risk factors. These results emphasise the importance of correct treatment to improve survival in autoimmune diabetes in adults.

*Supported by: Swedish Council for Working Life and Social Research*

## OP 29 Genetics of type 1 and type 2 diabetes

169

### A strategy for combining minor genetic susceptibility genes to improve prediction of disease in type 1 diabetes

C. Winkler<sup>1,2</sup>, J. Krumsiek<sup>3</sup>, J. Lempainen<sup>1,4</sup>, P. Achenbach<sup>1,2</sup>, H. Grallert<sup>5</sup>, E. Giannopoulos<sup>1,2</sup>, M. Bunk<sup>1,2</sup>, F.J. Theis<sup>3</sup>, E. Bonifacio<sup>6,2</sup>, A.G. Ziegler<sup>1,2</sup>;

<sup>1</sup>Institute of Diabetes Research, Helmholtz Zentrum München, Klinikum rechts der Isar, Technische Universität München, Neuherberg, Germany, <sup>2</sup>Forschergruppe Diabetes e.V., Neuherberg, Germany, <sup>3</sup>Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany, <sup>4</sup>Immunogenetics Laboratory, and Department of Pediatrics, University of Turku, Finland, <sup>5</sup>Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany, <sup>6</sup>Center for Regenerative Therapies, Dresden University of Technology, Germany.

**Background and aims:** Genome-wide association studies have identified gene regions associated with the development of type 1 diabetes (T1D). With the exception of the HLA and the INS gene regions, T1D susceptibility associated with any single loci is relatively small and these single gene regions have provided relatively little improvement in disease prediction. Aim of this study was to determine whether and how the combined allele frequency of non-HLA susceptibility genes can stratify islet autoimmunity and/or T1D risk in children with *a priori* familial diabetes risk.

**Material and methods:** The single nucleotide polymorphisms (SNPs) in 12 T1D susceptibility gene regions (*ERBB3*, *PTPN2*, *IFIH1*, *PTPN22*, *KIAA0350*, *CD25*, *CTLA4*, *SH2B3*, *IL2*, *IL18RAP*, *IL10*, *COBL*) were analyzed in 1290 children from the German BABYDIAB study, a prospective observational study that follows offspring of mothers or fathers with T1D from birth for the development of islet autoantibodies and T1D (median follow-up, 14 years). The sum of the scores for the 12 genes was assigned as the combined risk score for each child. Children were classified by their total number of risk alleles and risk stratification models developed.

**Results:** Receiver Operator Curves analysis was performed for all possible 4095 gene combinations. Overall, gene combinations were more effective in discriminating T1D than islet autoimmunity ( $P < 0.0001$ ) and most effective in children with an *a priori* high risk HLA genotype ( $P < 0.0001$ ). Highest discrimination (AUC, 0.73;  $P < 0.00001$ ) was obtained by the sum of risk alleles for 8 genes (*IFIH1*, *CTLA4*, *PTPN22*, *IL18RAP*, *SH2B3*, *KIAA0350*, *COBL*, *ERBB3*) using T1D as outcome in the HLA risk children. Categorizing risk allele score from these 8 genes in low, moderate and high (scores:  $< 6$ ,  $6-9$ ,  $> 9$ ) was able to stratify the risk for developing islet autoantibody (by age 14 years: low scores 0%; moderate scores 20%; high scores 37%;  $P = 0.001$ ) and for progression from islet autoantibody positivity to T1D (by age 10 years: moderate scores 40%; high scores 80%  $P = 0.03$ ). Overall T1D risk by age 14 years ranged from 0% in HLA risk children with low risk allele scores to 26.9% (95% CI, 15.2–38.6%;  $P < 0.0001$ ) in children with high risk allele scores.

**Conclusion:** Genotyping at multiple susceptibility T1D loci in children from affected families can identify neonates with sufficient risk to be considered for early intervention.

*Supported by: BMBF (KKNDM), JDRE, DFG-Research Center for Cluster of Excellence*

170

### New genetic loci associated with increased urinary albumin excretion rate in subjects with type 1 diabetes

N.K.A. Sandholm<sup>1,2</sup>, V.-P. Mäkinen<sup>1,3</sup>, C. Forsblom<sup>1,3</sup>, V. Harjutsalo<sup>1,4</sup>, M. Parkkonen<sup>1,3</sup>, L.M. Thorn<sup>1,3</sup>, J. Wadén<sup>1,3</sup>, M. Saraheimo<sup>1,3</sup>, B. He<sup>5</sup>, A.-M. Österholm<sup>3</sup>, K. Tryggvason<sup>5</sup>, P.-H. Groop<sup>1,3</sup>, FinnDiane Study Group; <sup>1</sup>Folkhälsan Institute of Genetics, Helsinki, Finland, <sup>2</sup>Department of Biomedical Engineering and Computational Science, Aalto University School of Science, Espoo, Finland, <sup>3</sup>Division of Nephrology, Helsinki University Central Hospital, Finland, <sup>4</sup>Dept Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland, <sup>5</sup>Dept Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.

**Background and aims:** Diabetic nephropathy is a major complication of diabetes and is often first manifested by the detection of an abnormal urinary

albumin excretion rate (AER). AER in diabetes is known to have an inherited component, but the associated genetic variants remain unknown. The aim of this study is to detect common genetic variation associated with elevated AER in patients with type 1 diabetes (T1D).

**Materials and methods:** This study includes 1925 subjects from the Finnish Diabetic Nephropathy (FinnDiane) Study who have T1D duration at least 10 years and have 24h AER measurements and genome-wide association study (GWAS) data available. Patients with either end-stage renal disease, or T1D duration below 15 years and persistent normoalbuminuria, were excluded. Genotypes for 550,000 single nucleotide polymorphisms were obtained from a GWAS performed on 3652 T1D subjects, and were extended to 2.4 million SNPs after imputation (MACH software, HapMap II CEU reference population). AER was  $\log_{10}$  transformed and analysed as a continuous trait to better describe the variation of the phenotype. As the use of anti-hypertensive medication strongly affects the AER, subjects with ( $n=1002$ ) and without ( $n=923$ ) anti-hypertensive medication were analysed separately. Linear regression was performed with PLINK software and the models were adjusted for sex, age at onset of T1D, duration of T1D, and the ten first principal components calculated with EIGENSTRAT software. The association results of the two groups were subsequently combined together with fixed effect meta-analysis.

**Results:** Five SNPs on chromosome 4q34 were genome-wide significantly ( $P<5\times 10^{-8}$ ) associated with elevated AER (smallest  $P=1.5\times 10^{-9}$ ). Furthermore, a total of 62 genetic regions reached  $P<1\times 10^{-4}$  and were selected for follow-up genotyping in order to detect further possible loci associated with elevated AER. Additional genotyping is currently ongoing in 3300 T1D subjects with Caucasian origin. Gene enrichment analysis conducted with MAGENTA software indicated that the association analysis results were enriched with genes involved in natural killer cell mediated immunity. **Conclusion:** We have identified a locus on chromosome 4q34 that is genome-wide significantly associated with high AER in T1D, as well as 62 putative susceptibility loci for high AER with  $P<1\times 10^{-4}$ . Replication in other T1D cohorts will tell whether these loci harbor true susceptibility genes for high AER or not.

Supported by: Folkhälsan Research Foundation, W. and E. Stockmann Foundation, SUMMIT

## 171

### A rare GLIS3 variant associated with resistance to type 1 diabetes

T. Awata<sup>1</sup>, H. Yamashita<sup>1</sup>, S. Kurihara<sup>1</sup>, T. Morita-Ohkubo<sup>1</sup>, Y. Miyashita<sup>2</sup>, E. Kawasaki<sup>3</sup>, H. Ikegami<sup>4</sup>, S. Tanaka<sup>5</sup>, T. Maruyama<sup>6</sup>, A. Shimada<sup>7</sup>, K. Takahashi<sup>8</sup>, Y. Kawabata<sup>4</sup>, T. Kobayashi<sup>2</sup>

<sup>1</sup>Department of Endocrinology and Diabetes, Saitama Medical University,

<sup>2</sup>Division of RI Laboratory, Biomedical Research Center, Saitama Medical

University, <sup>3</sup>Department of Metabolism/Diabetes and Clinical Nutrition,

Nagasaki University Hospital of Medicine and Dentistry, <sup>4</sup>Department of Endocrinology, Metabolism and Diabetes, Kinki University School of Medicine, Osaka, <sup>5</sup>Third Department of Internal Medicine, Interdisciplinary Graduate School of Medical and Engineering, University of Yamanashi,

<sup>6</sup>Department of Internal Medicine, Saitama Social Insurance Hospital,

<sup>7</sup>Department of Internal Medicine, Saiseikai Central Hospital, Tokyo,

<sup>8</sup>Department of Diabetes and Metabolism, Iwate Medical University, Morioka, Japan.

**Background and aims:** A number of novel non-HLA type 1 diabetes (T1D) susceptibility loci have been identified in Caucasians based on genome-wide association studies using common SNPs, whereas rare resistant variants have been identified in IFIH1. We previously reported the associations of INS, CTLA4, IL2RA, ERBB3 and CLEC16A with Japanese T1D. In the present study, we analyzed rare variants of the T1D candidate genes.

**Materials and methods:** We analyzed 706 T1D patients and 863 control subjects in Japan; the mean age at onset was 31.5 years. First, by next-generation sequencing, we screened 24 candidate genes, including IFIH1, WFS1 and GLIS3, in 96 T1D patients and 96 control subjects to identify any variations of the exons. Four DNA pools (2 x 48 patients and 2 x 48 controls) were prepared, and all exons of the 24 genes were PCR-amplified, then the DNA samples were mixed and sequenced with an Illumina Genome Analyzer IIx. The obtained sequence reads were mapped to the genes to identify variations. We next assessed their association in a large number of subjects recruited from the collaborating institutions.

**Results:** In total, 57 non-synonymous SNPs (nsSNPs) were discovered in 18 genes of the 24 genes studied. Approximately two-thirds of the identified nsSNPs appeared to be rare variants (MAF  $<0.05$  in control subjects). Among them, we further analyzed rare variants in IFIH1 and GLIS3. Three rare vari-

ants, R705S, N930S and rs3573203 (IVS14+1), were identified in IFIH1; two of which (R705S and N930S) have not yet been reported in Caucasians. The T allele of the rare rs3573203, reported to be associated with T1D resistance in Caucasians, did not significantly decrease in the T1D patients, although the MAF in the control subjects was higher in Japanese than in Caucasians (0.0351 vs 0.0093). The R705S and N930S variants were not significantly associated with T1D. Regarding GLIS3, four rare variants, H669R, S738F, A753V and R763H, were identified. The frequencies of these rare variants were all lower in the T1D patients in comparison to the controls, and A753V was significantly associated with resistance to T1D (absent in the T1D patients vs 0.0070 in the controls,  $P=0.0008$ ).

**Conclusion:** In the present study, a rare variant in GLIS3 was found to be protective against T1D. GLIS3, highly expressed in pancreatic beta cells, encodes a zinc finger transcription factor, which directly regulates insulin gene expression. The precise mechanism responsible for the association is unclear, but the variant may affect autoimmunity to insulin or to the GLIS3 protein itself.

## 172

### Comprehensive evaluation of type 2 diabetes susceptibility loci in the Japanese population by using 1000 genomes project data

H. Kazuo<sup>1</sup>, F. Hayato<sup>1</sup>, T. Johnson<sup>2</sup>, T. Yamauchi<sup>1</sup>, S. Maeda<sup>2</sup>, T. Tsunoda<sup>2</sup>, Y. Nakamura<sup>2</sup>, T. Kadowaki<sup>1</sup>

<sup>1</sup>University of Tokyo, <sup>2</sup>RIKEN, Yokohama, Japan.

**Background and aims:** Although over 50 type 2 diabetes (T2D) loci have been identified through genome-wide association studies (GWAS), the vast majority of the genetic predisposition to T2D still remains to be clarified. One explanation is that despite the use of high-throughput genotyping arrays, only a small proportion of genetic variants in the human genome are actually surveyed, especially in non-European populations.

**Materials and methods:** We explored the comprehensive catalog of genomic variations provided by the 1000 Genomes Project to identify variations conferring susceptibility to T2D in the Japanese population that were not detected in the previous scans. We imputed 10,524,368 variants derived from 286 East Asian subjects (November 2010 Release) in 5,976 cases and 20,829 controls genotyped by 610K single-nucleotide polymorphism (SNP) array.

**Results:** Overall concordance was good, although imputed SNPs with minor allele frequency (MAF) below 1% apparently contained poorly imputed SNPs. We then tested associations for T2D before and after adjusting for age, sex, and body mass index. We found that in addition to variants of the previously reported loci there were 25 loci harboring multiple variants with a p-value lower than  $10^{-5}$ . The MAF range was 0.01 to 0.45, and the odds ratios were between 1.10 and 1.48. We are conducting a replication study to confirm the association in another 7,000 cases and 3,500 controls. We also sought to define the most relevant SNPs for susceptibility to T2D in the previously identified genes. We did not find any stronger association with T2D than the originally reported SNPs in our population. However, there is a tendency that lower frequency variants were enriched in those with large effect size.

**Conclusion:** Our study highlights the benefits of using data derived from next-generation sequencing of the human genome such as the 1000 Genomes Project to explore T2D loci more comprehensively.

## 173

### The association of the mitochondrial DNA 16184-16193 poly-C variant with type 2 diabetes

C. Langenberg<sup>1</sup>, Z. Ye<sup>1</sup>, C. Gillson<sup>1</sup>, M. Plotka<sup>2</sup>, K.-T. Khaw<sup>3</sup>, J. Poulton<sup>2</sup>, N.J. Wareham<sup>1</sup>

<sup>1</sup>MRC Epidemiology Unit, Cambridge, <sup>2</sup>University of Oxford, <sup>3</sup>Department of Public Health and Primary Care, University of Cambridge, UK.

**Background and aims:** The association between the mitochondrial DNA 16181-16193 polycytosine (poly-C tract) that maps precisely to the OriB origin of replication, also known as the OriB or 16189 variant, and type 2 diabetes (T2D) has not been reliably characterized with studies providing conflicting results. We performed a meta-analysis of results from a large new case-control study and the published evidence to systematically investigate the association between the 16189 variant and T2D.

**Materials and methods:** We genotyped the 16189 variant by pyrosequencing followed by Sanger cycle sequencing for all individuals with a homopolymeric C-tract sequence  $\geq 10$  in the Norfolk Diabetes Case-Control Study (NDCCS), which comprised 5,419 T2D cases and 6,738 population-based

controls. We performed a systematic review identifying all studies of the 16189 variant and T2D published prior to September 2011. We used random effects meta-analysis methods to combine results from 19 published studies and the NDCCS including a total of 16,060 T2D cases and 17,783 controls, and examined potential sources of heterogeneity.

**Results:** In a combined analysis, the pooled odds ratio for T2D of the 16184-16193 poly-C tract was 1.31 (95% confidence interval (CI): 1.18–1.46). Sample size and ethnic background of study participants were identified as significant sources of heterogeneity between studies. Analyses restricted to larger studies with 500 or more T2D cases showed an odds ratio of 1.15 (95% CI: 1.05–1.25). In ethnic-group stratified analyses, the odds ratio for the association of the 16189 variant with T2D was 1.10 (95%CI: 1.01–1.20) in Europeans and 1.49 (95%CI: 1.27–1.74) in Asians.

**Conclusion:** Results from this systematic review and meta-analysis suggests that the mitochondrial DNA 16184-16193 poly-C tract is associated with an increased risk of type 2 diabetes.

## 174

### Functional analysis of GCKR mutations identified in subjects with hypertriglyceridaemia demonstrates the inherent challenges in clinical interpretation of rare variants

A. Raimondo<sup>1</sup>, M.G. Rees<sup>1,2</sup>, J. Wang<sup>3</sup>, A. Barrett<sup>1</sup>, F.S. Collins<sup>2</sup>, R.A. Hegele<sup>3</sup>, A.L. Gloyne<sup>1,4</sup>

<sup>1</sup>Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, UK, <sup>2</sup>National Human Genome Research Institute, National Institute of Health, Bethesda, USA, <sup>3</sup>Robarts Research Institute, University of Western Ontario, London, Canada, <sup>4</sup>Oxford NIHR Biomedical Research Centre, Churchill Hospital, UK.

**Background and aims:** The clinical interpretation of rare non-synonymous variants identified via sequencing projects is currently a major challenge. GCKR encodes glucokinase regulatory protein (GKRP), a predominantly nuclear protein that inhibits hepatic glucokinase (GCK). This inhibition is modulated by fructose-1-phosphate (F1P) and fructose-6-phosphate (F6P). Recent genetic and functional studies have demonstrated that GCKR variants across the allelic spectrum have effects on glucose and lipid levels, but the clinical interpretation of rare variants identified in patients with extreme lipid phenotypes has not been explored. The aim of this study was to use an established suite of functional assays to determine the cell biological and biochemical attributes of twelve non-synonymous GCKR variants identified in individuals with hypertriglyceridaemia, hypothesising that these variants would show functional characteristics distinct from those identified in individuals with less extreme phenotypes.

**Materials and methods:** All twelve variants were transiently transfected into HeLa cells as fluorescent fusion plasmids and analysed for their subcellular localisation relative to wild type (WT) GKRP. Nine variants were expressed and purified from *E. coli* and assayed for their ability to inhibit glucose-stimulated GCK activity in the presence and absence of F1P and F6P. *In silico* analysis of all variants was assessed using SIFT and PolyPhen 2.

**Results:** Of the twelve GCKR variants studied, nine, including M344I, showed nuclear localisation and expression levels similar to that of WT GKRP. Three variants, including R259W, showed near-complete loss of nuclear localisation. Kinetic characterisation of nine variants, selected on the basis of their proximity to conserved sugar binding motifs, demonstrated that four showed altered kinetics. R259W GKRP showed reduced GCK inhibition ( $p < 0.001$  compared to WT GKRP) and significantly decreased interaction with both F6P ( $p < 0.001$ ) and F1P ( $p < 0.05$ ). M344I GKRP showed no difference in response to F6P ( $p > 0.1$ ) but exhibited reduced GCK inhibition and interaction with F1P ( $p < 0.001$ ). R259W and M344I showed the largest loss of response to F6P and F1P, respectively, of any human variant GKRP tested to date. *In silico* analysis did not provide a consensus on the predicted pathogenicity of the majority of the twelve variants, including M344I.

**Conclusion:** Our results indicate that GCKR variants that contribute to hypertriglyceridaemia cause substantial functional defects. Individual variants differ in their cellular and kinetic effects, supporting the need to assess both when evaluating the pathogenicity and clinical significance of rare GCKR variants.

Supported by: Wellcome Trust 095101/Z/10/Z

## OP 30 Beta cell function in vivo

### 175

#### Hepatocyte-derived factor(s) drive beta cell hyperplasia in insulin resistant mice

A. El Ouaamari, D. Kawamori, E. Dirice, C. Liew, J. Shadrach, J. Hu, H. Katsuta, J. Hollister-Lock, A. Wagers, R. Kulkarni; Joslin Diabetes Center, Boston, USA.

Inter-organ communication controls key aspects of energy homeostasis and its dysregulation may underlie metabolic disorders such as diabetes. Islet  $\beta$ -cell hyperplasia occurs as an adaptive response to physiological (pregnancy) or pathophysiological (obesity) insulin resistant states. To test the hypothesis that cross-talk between liver and pancreatic islets mediates  $\beta$ -cell growth in response to insulin resistance, we used the Liver-specific Insulin Receptor Knockout (LIRKO) mouse, a unique model that exhibits a dramatic islet hyperplasia. To explore whether circulating factors in LIRKO mice promote  $\beta$ -cell proliferation, we established *in vivo* transplantation and parabiotic mouse models and assessed  $\beta$ -cell replication by bromodeoxyuridine incorporation. We also cultured mouse or human islets for 48 hours in serum LIRKO or controls, followed by confocal microscopic evaluation of  $\beta$ -cell growth by Ki67 immunostaining. Our studies reveal that: (1) control mice parabiosed to LIRKO mice show a 4-fold increase in  $\beta$ -cell mitosis and (2) control islets transplanted under the kidney capsule in LIRKO mice exhibit 7-fold increased  $\beta$ -cell proliferation. Furthermore, LIRKO serum enhanced  $\beta$ -cell proliferation in primary mouse islets, control human islets, and in islets from a patient with type 2 diabetes. Finally, culturing mouse islets in conditioned media from liver explants (LECM) or hepatocytes (HCM) for 24 h enhanced  $\beta$ -cell growth respectively. Taken together, these data indicate the existence of circulating, non-neural and non-cell autonomous  $\beta$ -cell growth factors, and strongly implicate the liver as a critical source of these novel  $\beta$ -cell growth factor(s). The enhanced proliferation observed in  $\beta$ -cells from both control and diabetic humans underscores the significance of our model to identify the putative  $\beta$ -cell growth factor(s) with the long term goal of increasing  $\beta$ -cell number in patients with diabetes.

Supported by: SFD, AFD, ADA, JDRF

### 176

#### Melatonin receptor 2 (MT2) knock-out mice display increased insulin release

C.L.F. Nagorny Holmberg<sup>1</sup>, H. Bennet<sup>2</sup>, L. Shcherbina<sup>3</sup>, M. Fex<sup>2</sup>, N. Wierup<sup>3</sup>, H. Mulder<sup>1</sup>

<sup>1</sup>Molecular Metabolism, <sup>2</sup>Diabetes and Celiac Disease, <sup>3</sup>Neuroendocrine Cell Biology, Clinical Sciences Malmö, Sweden.

**Background and aims:** Numerous independent studies have confirmed our initial finding that a variant of the melatonin receptor 1B (*MTNR1B* (MT2)) gene is associated with increased fasting plasma glucose, impaired early insulin secretion, and Type 2 Diabetes Mellitus (T2DM). Both melatonin receptors are expressed in human islets. Given the increased mRNA expression of *MTNR1B* in islets of individuals carrying the risk variant, the pathogenetic effects could be exerted via a direct inhibitory effect of melatonin on  $\beta$ -cells. This is based on the notion that melatonin, in most studies, appears to inhibit insulin secretion. To address this hypothesis, we examined glucose homeostasis *in vivo* and *in vitro* in mice lacking the *MTNR1B*/MT2 receptor.

**Materials and methods:** Whole body female knock out mice for the MT2 receptor were kindly provided by Professor David Weaver. IVGTTs as well as hyperinsulinemic-euglycemic clamps were performed to investigate  $\beta$ -cell function and whole body glucose metabolism in mice at 12 weeks of age. The number of islets and  $\beta$ -cell mass were investigated by immunohistochemistry and morphometry (12–14 weeks, male). Static 1 h incubations of isolated islets were performed in 12 week old female mice. Statistical analysis was by Student's *t*-tests; mean  $\pm$  standard errors of the mean (SEM) are given.

**Results:** After intravenous injection of glucose MT2 mice showed lower plasma glucose (AUC: MT2: 556.7  $\pm$  37.3; WT: 694.1  $\pm$  54.5; arbitrary units (AU);  $p=0.057$ ) as well as elevated insulin (AUC: MT2: 205.2  $\pm$  28.1; WT: 91.2  $\pm$  6.1 AU;  $p<0.02$ ). This was also reflected by the acute insulin response (AIR; MT2: 86.3  $\pm$  14.2; WT: 21.7  $\pm$  3.1;  $p<0.02$ ). Glucose elimination (K: %/min) was not different in MT2 and WT mice. The clamp revealed an insulin-resistant phenotype in MT2 mice. The glucose infusion rate was lower in MT2 (MT2: 5.5  $\pm$  1.3; WT: 10.9  $\pm$  1.8 mg/kg/min;  $p=0.03$ ), while hepatic glucose



production during the clamp was higher (MT2:  $1.0 \pm 1.6$ ; WT:  $-6.0 \pm 2.1$  mg/kg/min;  $p < 0.02$ ). There was no difference in islet size, but the number of islets was increased in MT2 (MT2:  $2.5 \times 10^{-6} \pm 3.2 \times 10^{-7}$ ; WT:  $1.3 \times 10^{-6} \pm 1.7 \times 10^{-7}$  AU;  $p < 0.01$  (Mann Whitney, U-test)), resulting in an increased  $\beta$ -cell mass. *In vitro* batch incubations agreed with *in vivo* data: MT2 islets showed improved glucose-stimulated insulin secretion (GSIS) (MT2:  $4.5 \pm 0.3$ ; WT:  $3.0 \pm 0.2$  (Fold change over basal ng/islet/h);  $p < 0.001$ ).

**Conclusion:** The hyperinsulinemic phenotype of the MT2 mice *in vivo* and *in vitro* confirms our genetic data, where the risk genotype displayed decreased first phase insulin secretion and decreased glucose tolerance due to a possible gain of function mutation. Our mice, having a loss of function, display the opposite phenotype: they release more insulin and tolerate glucose better after an intravenous glucose challenge. The reduced insulin sensitivity during the clamp may be an adaptation to this improved capacity to secrete insulin.

Supported by: EFSD/MSD grant

## 177

### The GLP-1-gastrin dual agonist ZP3022 increases beta cell mass via an increase in mean islet mass in db/db mice

D.L.C. Almholt<sup>1</sup>, L.S. Dalbøge<sup>2</sup>, J.L. Tolborg<sup>1</sup>, C. Grøndahl<sup>1</sup>, K. Fosgerau<sup>1</sup>, T.S.R. Neerup<sup>1</sup>;

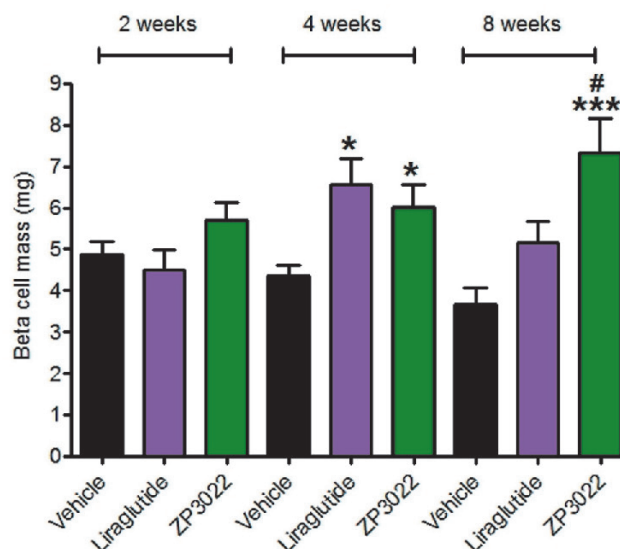
<sup>1</sup>Pharmacology, Zealand Pharma A/S, Glostrup, <sup>2</sup>Gubra ApS, Hørsholm, Denmark.

**Background and aims:** Combination treatment of diabetic db/db mice with exendin-4 and gastrin has previously been shown to preserve beta-cell mass by stimulating beta-cell growth and differentiation. Here we investigate the effects of a novel and potent GLP-1-gastrin receptor dual agonist (ZP3022, invented by Zealand Pharma A/S) that acts on the GLP-1 and CCKB (gastrin) receptors.

**Materials and methods:** Equimolar doses ( $2 \times 50$  nmol/kg/day) of ZP3022, liraglutide, or vehicle were administered by twice daily subcutaneous injection to male db/db mice for 2, 4 or 8 weeks. HbA1c and fasting blood glucose levels were determined at treatment start and at termination. Pancreata were removed and fixed in phosphate buffered formaldehyde for stereological determination of beta-cell mass. Furthermore, the total number of islets, mean islet mass, and number of proliferating beta cells (insulin and ki67 double immunoreactive cells) were determined stereologically in the 8 weeks treatment groups.

**Results:** After 2, 4, and 8 weeks of treatment, both ZP3022 and liraglutide caused a significant improvement in glycemic control as measured by HbA1c (8 weeks: Vehicle,  $7.4 \pm 0.5\%$ ; Liraglutide,  $5.3 \pm 0.4\%$ ; ZP3022,  $5.6 \pm 0.6\%$ ,  $p < 0.001$  for ZP3022 and liraglutide vs. vehicle) as well as fasting blood glucose levels. Both ZP3022 and liraglutide caused a significant increase in beta-cell mass compared to vehicle following 4 weeks of treatment (Figure,  $p < 0.05$ ). However, after 8 weeks of treatment, only ZP3022 resulted in a significant increase in beta-cell mass, compared to both vehicle- and liraglutide-treated mice (Figure,  $p < 0.001$  and  $p < 0.05$ , respectively). Following 8 weeks of treatment, neither the liraglutide- nor the ZP3022-treated groups showed any difference in the total number of islets compared to vehicle. In contrast, the mean islet mass was significantly increased in the ZP3022-treated group compared to vehicle ( $p < 0.01$ ). Finally, the total number of proliferating (ki67 positive) beta cells was significantly increased compared to vehicle in both the liraglutide- ( $p < 0.05$ ) and the ZP3022-treated ( $p < 0.001$ ) groups.

**Conclusion:** The study demonstrates that treatment of db/db mice with the GLP-1-gastrin receptor dual agonist ZP3022 causes a sustained improvement in glycemic control accompanied by an increase in the beta-cell mass. In contrast, liraglutide displays a transient effect on beta-cell mass. Moreover, it is demonstrated that the increased beta-cell mass is related to an increased proliferation of beta cells and an increased mean islet mass.



## 178

### Metreleptin therapy in leptin-deficient patients with lipodystrophic syndromes: effects on insulin secretion

C. Vatiér<sup>1,2</sup>, S. Fetita<sup>3</sup>, P. Boudou<sup>4</sup>, C. Tchankou<sup>5</sup>, L. Deville<sup>6</sup>, J.-F. Gautier<sup>7,3</sup>, C. Vigouroux<sup>1,2</sup>;

<sup>1</sup>INSERM UMRS\_938, <sup>2</sup>UPMC Université Paris 06, <sup>3</sup>Service de diabétologie et d'Endocrinologie, AP-HP Hôpital Saint-Louis, <sup>4</sup>Service de Biochimie et d'Hormonologie, AP-HP Hôpital Saint-Louis, <sup>5</sup>AP-HP Hôpital Saint-Louis, <sup>6</sup>Pharmacie, AP-HP Hôpital Saint-Louis, <sup>7</sup>INSERM UMRS\_872, Paris, France.

**Background and aims:** Lipodystrophic syndromes (LD) are genetic or acquired diseases characterized by total or partial body fat loss with severe alterations of lipid and glucose homeostasis. Although major insulin resistance is the prominent metabolic feature in LD, leading to severe difficulties to control diabetes, the progressive deterioration of glucose control is also linked to insulin secretion defects. The correction of leptin deficiency associated with LD has been shown to improve insulin sensitivity by decreasing hepatic and muscular lipotoxicity. However, only a few studies have investigated its effects on insulin secretion. We took advantage of a compassionate program of recombinant methionyl human leptin (metreleptin) therapy in patients with LD complicated with diabetes to study the effects of metreleptin on insulin secretion.

**Materials and methods:** Fifteen non HIV-infected patients with LD and leptin deficiency (4 generalized and 11 partial) are currently treated by a daily subcutaneous metreleptin injection ( $0.04$  to  $0.12$  mg/kg/d), gracefully supplied through a compassionate program authorized by the French Health Regulatory Agency (AFSSAPS). A favorable effect of metreleptin on glycemic control was defined as (1) a 0.5-point decrease in HbA1c, or (2) HbA1c stability with a decrease of  $>20\%$  in insulin doses or  $>50\%$  in oral antidiabetic drugs doses or discontinuation of one antidiabetic drug class, between treatment initiation (D0) and last visit. The first phase of insulin secretion was evaluated by (1) the Insulinogenic Index from 0 to 30 min of an Oral Glucose Tolerance Test ( $\Delta$  insulinemia (0-30) /  $\Delta$  glycemia (0-30)) at D0, after 30 days (M1, 10 patients) and one year (M12, 6 patients) of treatment, and (2) the acute insulin response to glucose (AIR) measured during a hyperglycemic clamp, at D0 and after one year of therapy (M12, 7 patients).

**Results:** Fourteen patients were studied (one patient interrupted her treatment), with a follow-up of  $15.4 \pm 9.6$  months (mean  $\pm$  SD). At the last visit, glycemic control was improved in 11 patients; weight loss was  $3.4 \pm 3$  kg; nine of the 12 patients with hypertriglyceridemia and 9 of the 11 patients with elevated liver enzymes at D0 significantly improved these alterations after metreleptin therapy. The Insulinogenic Index significantly increased in 8 of 10 patients at M1 and in 4 of 6 patients at M12 (median increase 374.7% range 86.3-1709.5,  $p = 0.04$  and 149.1% range 43.5-399%,  $p = 0.03$  at M1 and M12, respectively). AIR increased in 6 of 7 patients at M12 (median increase 246.1% range 96.6-900%,  $p = 0.07$ ). The 2 patients who had the better improvement of insulin secretion had the same genetic disease (AGPAT2 mutation) and the metabolic response to metreleptin was greater in patients with general-

ized lipodystrophy syndrome. The patients who did not improve their insulin secretion had partial lipodystrophy.

**Conclusion:** These results suggest that improvement of insulin secretion contributes to the metabolic efficiency of metreleptin therapy in most patients with lipodystrophic syndromes and diabetes.

Supported by: Amylin Pharmaceuticals, Inc. INSERM. DHOS. CODDIM

## 179

### Lowering GIP secretion has beneficial role in reducing obesity and insulin resistance without impairing glucose tolerance and osteogenesis

D. Nasteska, N. Harada, S. Yamane, K. Suzuki, A. Hamasaki, E. Joo, K. Sasaki, K. Shibue, T. Harada, Y. Nakamura, N. Inagaki;

Department of Diabetes and Clinical Nutrition, Kyoto University, Japan.

**Background and aims:** Incretin hormones, such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), represent the backbone of the enteroinsular axis. GIP has pancreatic and extrapancreatic effects implicated in beta cells function, bone formation and adipose tissue modification. Previous animal studies have shown changes occurring in absence of GIP receptor signalling, but knowledge regarding reduced GIP secretion remains insufficient. Hence, we have generated GIP-deficient mice with reduced and lacking GIP secretion.

**Materials and methods:** We generated GIP-deficient mice with truncated GIP gene. 9-weeks old wild type (WT), GIP-gene reduced, heterozygous mice (GIP<sup>+/-</sup>) and GIP-gene lacking, homozygous mice (GIP<sup>-/-</sup>) underwent oral glucose tolerance test (OGTT) in which blood glucose, insulin and GIP levels were measured. Furthermore, islets were isolated and assessed for insulin release during glucose and GIP stimulation. GIP-receptor (GIPR) levels were assessed by semiquantitative RT-PCR. In addition, tibiae were isolated and prepared for bone histomorphometry assessment. Another set of these groups of mice was kept on control diet (with 10% of fat) and high-fat diet (with 60% of fat) for 8 weeks, starting from 8 weeks of age. Afterwards, OGTT and insulin tolerance test (ITT) were performed. Finally, total body fat was measured by CT scan.

**Results:** During OGTT, GIP<sup>+/-</sup> mice showed significantly decreased GIP levels (~50%) compared to WT. In GIP<sup>-/-</sup> mice, GIP levels were below detectable range. Insulin levels at 15 min of OGTT were significantly lower in GIP<sup>+/-</sup> and GIP<sup>-/-</sup> mice compared to WT. Islets from GIP<sup>+/-</sup> and GIP<sup>-/-</sup> mice showed increased insulin secretion upon stimulation by 11.1 mM glucose and GIP similarly to WT islets. No statistical significance of GIPR mRNA expression occurred in pancreatic islets among all groups. Bone histomorphometry revealed that in GIP<sup>+/-</sup> mice bone volume, trabecular number and thickness, and osteoclast surface did not statistically differ compared to WT. In GIP<sup>-/-</sup> mice, however, bone volume, trabecular number and thickness were significantly decreased while osteoclast surface was statistically increased. OGTT after high-fat diet showed no significant changes in blood glucose levels between WT and GIP<sup>+/-</sup> mice whereas GIP<sup>-/-</sup> mice had significant increase compared to WT. GIP<sup>+/-</sup> and GIP<sup>-/-</sup> mice had significantly lower insulin levels than WT. ITT showed significant improvement of insulin sensitivity in GIP<sup>+/-</sup> and GIP<sup>-/-</sup> mice compared to WT. Total body fat was significantly decreased in GIP<sup>+/-</sup> mice (1.3-fold) compared to WT; GIP<sup>-/-</sup> mice had significantly lowest total body fat. Expression levels of GIPR in white and brown adipose tissue in GIP<sup>+/-</sup> mice were similar to those in WT while GIP<sup>-/-</sup> mice showed significant increase compared to WT.

**Conclusion:** Reduced GIP secretion, as shown in our GIP<sup>+/-</sup> mice model, does not seem to affect the function of GIPR in beta cells, or bone formation and volume. During metabolic stress, such as high-fat diet feeding, decreased GIP levels lead to decrease in insulin secretion and fat accumulation in adipose tissue without impairing glucose tolerance. These results suggest that lowering GIP secretion may have beneficial role in reducing obesity without impairing the other functions of GIP.

## 180

### The effect of decreased insulin sensitivity and insulin secretion on cerebrospinal fluid insulin level: an analysis in normoglycaemic patients with Alzheimer's disease

M.V. Macesic<sup>1</sup>, N.M. Lalic<sup>1</sup>, V.S. Kostic<sup>2</sup>, A. Jotic<sup>1</sup>, E. Stefanova<sup>2</sup>, K. Lalic<sup>1</sup>, T. Milicic<sup>1</sup>, L. Lukic<sup>1</sup>, N. Rajkovic<sup>1</sup>, J. Seferovic-Mitrovic<sup>1</sup>, J. Stanarcic<sup>1</sup>;

<sup>1</sup>Clinic for endocrinology, <sup>2</sup>Clinic for neurology, Belgrade, Serbia.

**Background and aims:** Previous studies have shown that impairment in insulin sensitivity and insulin secretion play the important role in pathogenesis

of Alzheimer's disease (AD), together with the effect of the peripheral hyperinsulinemia on cerebrospinal fluids (CSF) insulin level. However, the relationship between decreased insulin sensitivity and insulin secretion with CSF insulin level in patients with AD has not yet been clarified. This study was aimed to evaluate correlation of possible changes in insulin sensitivity and insulin secretion capacity with CSF insulin level in normoglycemic patients with AD.

**Materials and methods:** The study included 63 normoglycemic patients with AD (group A; BMI: 24.73 +/- 0.78 kg/m<sup>2</sup>, age: 71.32 +/- 7.54 years), and 27 healthy matched controls (group B; BMI: 25.04 +/- 0.69 kg/m<sup>2</sup>, age: 64.56 +/- 6.83 years). Insulin sensitivity was estimated using euglycemic hyperinsulinemic clamp method, total glucose uptake (M value) was calculated as the amount of glucose infused during steady state period (80-120 min); acute insulin response (AIR) was calculated as the average increase in insulinemia compared to the basal value in the first 8 min of intravenous glucose tolerance test, plasma and CSF insulin level were determined by radioimmunoassay and plasma adiponectin level by ELISA.

**Results:** Evaluation of the insulin sensitivity assessment has shown significantly higher levels of insulin resistance (IR) in group A compared to group B (M value; A: 6.39 +/- 0.52; B: 8.18 +/- 0.56 mg/min/kg, p<0.01). Also, parameter of early-phase of insulin secretion, AIR, were significantly lower in group A compared to group B (A: 20.26 +/- 2.77; B: 67.19 +/- 5.30 mU/l, p<0.01). Simultaneously, basal plasma insulin levels were higher in group A vs group B (A: 15.63 +/- 1.78; B: 7.42 +/- 0.77 mU/l, p<0.01), while the plasma adiponectin levels were significantly lower in group A vs B (A: 15.23 +/- 0.75; B: 18.59 +/- 2.06 ng/ml, p<0.01). In addition, in group A, insulin sensitivity index, expressed as M value, significantly correlated with CSF insulin level (r=0.908, p<0.01), whereas no correlations were detected between insulin secretion and CSF insulin level (r=0.002, p=ns). However, we found the significant correlation among M value, AIR and CSF insulin level (p=0.001, p=0.038, respectively, R<sup>2</sup>=0.805, p<0.001).

**Conclusion:** Our results have demonstrated decreased both insulin sensitivity and insulin secretion level in normoglycemic patients with AD, as well as relationship with CSF insulin level. These results also imply that insulin resistance might exert its pathogenic effect on the occurrence of AD through hyperinsulinemia and decreased adiponectin level.

## OP 31 The importance of glycaemic control: results from large scale studies

181

### The association of HbA<sub>1c</sub> with risk of cardiovascular hospitalisations and all-cause mortality is U-shaped

G.A. Nichols<sup>1</sup>, S. Gotlib<sup>2</sup>, S. Parasuraman<sup>2</sup>;<sup>1</sup>Kaiser Permanente Center for Health Research, Portland, <sup>2</sup>AstraZeneca LP, Wilmington, USA.

**Background and aims:** A recent analysis of the UK General Practice Research Database found that both low and high mean glycosolated hemoglobin (HbA<sub>1c</sub>) values were associated with increased all-cause mortality and cardiac events. The objective of our study was to determine whether these findings could be confirmed in a US population

**Materials and methods:** The study site was Kaiser Permanente Northwest, a group model health maintenance organization located in the Northwestern United States that provides comprehensive medical care to approximately 475,000 members. Using electronic medical record data, we identified 26,636 members with type 2 diabetes and no known prior cardiovascular hospitalization. Beginning in 2002, we identified the earliest date patients had HbA<sub>1c</sub>, systolic blood pressure, and low density lipoprotein cholesterol measurements within 6 months of each other as the index date and followed them until they died, disenrolled, or 31 December 2010. The outcomes, analyzed separately, were a hospitalization with a 1st listed diagnosis of cardiovascular disease (CVDh) and all-cause mortality. We used mean HbA<sub>1c</sub> values between the index date and outcome or end of follow-up to stratify patients into levels of HbA<sub>1c</sub> control (<6%, 6-6.4%, 6.5-6.9%, 7-7.4%, 7.5-7.9%, 8-8.4%, 8.5-8.9%, ≥9%). We estimated incidence rates of CVDh and mortality independently associated with these strata, adjusted for age, sex, and duration of diabetes. We then constructed Cox regression models to estimate the hazard ratios for the strata controlling for age, sex, race, diabetes duration, blood pressure, lipids, comorbidities, body mass index, smoking, and prescription pharmacotherapy.

**Results:** Over mean (SD) follow-up of 5.6 (2.5) years, 7.3% of the total sample experienced a CVDh and 10.9% died. Adjusted CVDh incidence was lowest among patients with mean HbA<sub>1c</sub> 7.0-7.4% (9.3/1000 person-years, 95% CI 8.3-10.4) and was significantly higher among patients with mean HbA<sub>1c</sub> <6% (12.8, 11.1-14.7), 8.5-8.9% (14.3, 11.8-17.3) and ≥9% (17.9, 15.5-20.7). Mortality rates displayed a similar relationship, with the lowest rate occurring among patients with mean HbA<sub>1c</sub> 7.0-7.4% (8.7, 7.8-9.6) and significantly higher rates among those with HbA<sub>1c</sub> <6% (16.2, 14.5-18.0), 6-6.4% (12.4, 11.3-13.7), and ≥9% (14.4, 12.3-16.9). Relative to patients with HbA<sub>1c</sub> 7-7.4%, the fully adjusted Cox models showed significantly greater risk of CVDh among patients with mean HbA<sub>1c</sub> <6%, 6.0-6.4%, 6.5-6.9%, 8.5-8.9%, and ≥9% (table). Mortality risk was also significantly greater for patients with mean HbA<sub>1c</sub> <6%, 6.0-6.4%, 6.5-6.9%, and ≥9% relative to 7.0-7.4%.

**Conclusion:** The association of mean HbA<sub>1c</sub> with CVDh and all-cause mortality is U-shaped even after accounting for numerous other risk factors, suggesting that guidelines for glycemic control might need to include minimum HbA<sub>1c</sub> targets.

Adjusted Hazard Ratios for CVD Hospitalizations and All-Cause Mortality

Mean A1C	CVD Hospitalization			All-Cause Mortality		
	Hazard Ratio	95% CI	P Value	Hazard Ratio	95% CI	P Value
< 6% (n=2,464)	1.65	1.35-2.02	<0.001	1.73	1.49-2.01	<0.001
6.0-6.4% (n=4,355)	1.22	1.03-1.45	0.023	1.45	1.27-1.66	<0.001
6.5-6.9% (n=5,358)	1.18	1.01-1.38	0.039	1.21	1.07-1.38	0.003
7.0-7.4% (n=4,836)	1.00	REF	--	1.00	REF	--
7.5-7.9% (n=3,460)	1.06	0.89-1.27	0.505	0.93	0.80-1.09	0.383
8.0-8.4% (n=2,281)	1.03	0.84-1.28	0.755	0.99	0.83-1.19	0.903
8.5-8.9% (n=1,412)	1.49	1.17-1.89	0.001	1.12	0.89-1.42	0.337
≥ 9.0% (n=2,470)	1.68	1.37-2.07	<0.001	1.41	1.15-1.72	<0.001

Supported by: AstraZeneca

182

### Glycaemic treatment responders in real life have lower risk of cardiovascular disease and total mortality - an observational study from the Swedish NDR

K. Eeg-Olofsson<sup>1</sup>, B. Eliasson<sup>1</sup>, B. Zethelius<sup>2</sup>, A.-M. Svensson<sup>3</sup>, S. Gudbjörnsdottir<sup>1</sup>, J. Cederholm<sup>2</sup>;<sup>1</sup>Institute of Medicine, Gothenburg, <sup>2</sup>Department of Public Health and Caring Sciences, Uppsala, <sup>3</sup>Centre of Registers in Region Västra Götaland, Gothenburg, Sweden.

**Background and aims:** The relationship between glycaemic control and cardiovascular risk in type 2 diabetes (T2D) remains unclear. The aim of this observational study was to assess the association between improved glycaemic control during follow-up and risk for coronary heart disease (CHD), cardiovascular disease (CVD) and total mortality in patients with T2D from the Swedish National Diabetes Register.

**Materials and methods:** 18035 patients with baseline HbA<sub>1c</sub> 7-8.9%, aged 30-75 years and with no previous CVD, were identified in 2003-2006, and follow-up until December 2009 (mean 5.7 years). Two groups of patients were created based on the median for change in HbA<sub>1c</sub> during follow-up: 8923 patients (mean baseline age 62.0 years, diabetes duration 8.3 years) with HbA<sub>1c</sub> decreasing 0.1% or more from baseline to follow-up (mean baseline HbA<sub>1c</sub> 7.8±0.5% and final 7.0±0.6%), and 9112 patients (mean baseline age 61.3 years, duration 9.2 years) with stable or increasing HbA<sub>1c</sub>, more than or equal to 0% from baseline to follow-up (mean baseline HbA<sub>1c</sub> 7.7±0.5% and final 8.3±0.9%).

**Results:** Absolute risk of first-incident fatal/nonfatal CVD was 15.1 events per 1000 person-years in patients with decreasing HbA<sub>1c</sub> and 26.1 in patients with stable/increasing HbA<sub>1c</sub>. Adjusted hazard ratios at Cox regression analysis for first-incident fatal/nonfatal CHD, fatal/nonfatal CVD and total mortality, with decreasing HbA<sub>1c</sub> compared to stable/increasing HbA<sub>1c</sub>, were 0.61 (95% CI 0.53-0.69), 0.63 (0.56-0.70), and 0.56 (0.50-0.64), respectively, all p<0.001. Adjustment was made for a propensity score with stratification by octiles, including covariates age, sex, diabetes duration, baseline HbA<sub>1c</sub> and traditional CVD risk factors and treatments, as well as changes in these risk factors and treatments during the study period. Differences in included covariates between the two groups were balanced when adjusted with the propensity score.

**Conclusion:** In T2D patients in real life an HbA<sub>1c</sub> reduction of mean 0.8% from mean 7.8% to 7.0% (to the treatment target) was associated with substantially lower risk of CHD, CVD and total mortality, compared to patients with stable/increasing HbA<sub>1c</sub> of 7.7-8.3%.

183

### Effect of screening for type 2 diabetes on population mortality over ten years: the ADDITION-Cambridge cluster-randomised controlled trial

R.K. Simmons<sup>1</sup>, J.B. Echouffo-Tcheugui<sup>1</sup>, S.J. Sharp<sup>1</sup>, L.A. Sargeant<sup>1</sup>, K.M. Williams<sup>2</sup>, A.T. Prevost<sup>2</sup>, A.-L. Kinmonth<sup>2</sup>, N.J. Wareham<sup>1</sup>, S.J. Griffin<sup>1</sup>;<sup>1</sup>MRC Epidemiology Unit, <sup>2</sup>Department of Public Health and Primary Care, General Practice and Primary Care Research Unit, Cambridge, UK.

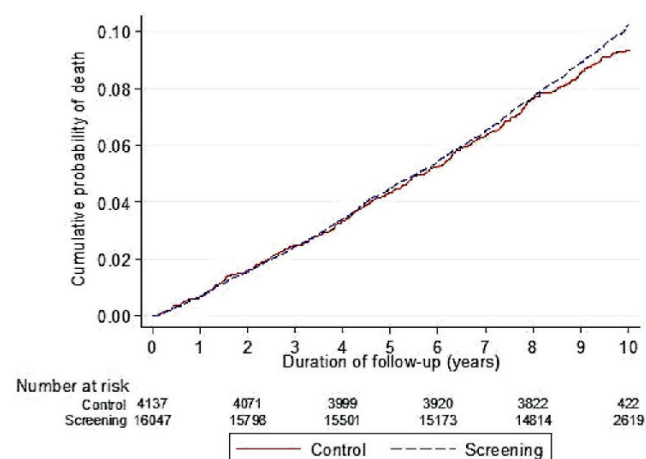
**Background and aims:** Uncertainty persists concerning the benefits of screening for type 2 diabetes. We assessed the impact of a population-based stepwise screening programme on mortality in a cluster-randomised trial.

**Materials and methods:** 33 practices in Eastern England were allocated in an unbalanced design to i) screening followed by intensive multifactorial treatment for detected diabetes (IT, n=15), ii) screening plus routine care according to national guidelines (RC, n=13) and iii) a no-screening control group (n=5). The study population comprised 20,184 individuals aged 40-69 years, at high risk of prevalent undiagnosed diabetes, based on a previously validated risk score. In screening practices, individuals were invited to a stepwise programme including random capillary blood glucose and HbA<sub>1c</sub> tests, a fasting capillary test, and a confirmatory oral glucose tolerance test. The primary analysis was by intention-to-screen and compared all-cause mortality rates between screening (IT and RC combined) and control groups after a median 9.6 years of follow-up, allowing for the cluster design.

**Results:** Of 16,047 high-risk individuals in screening practices, 15,089 (94%) were invited for screening during 2001-06, 11,737 (73.1%) attended and 466 (2.9%) were diagnosed with diabetes. 4,137 control individuals were followed-up. During 184,057 person-years of follow-up, there were 1,532 deaths in the screening practices and 377 in control practices (mortality hazard ratio [HR]: 1.06, 95% CI 0.90,1.25) (Figure One). There was no significant



reduction in cardiovascular (HR: 1.02, 95% CI 0.75,1.38), cancer (HR: 1.08, 95% CI 0.90,1.30) or diabetes-related mortality (HR: 1.26, 95% CI: 0.75,2.10) associated with invitation to screening. Compared with the control group, screening attenders had a lower mortality (HR: 0.69, 95%CI 0.59-0.80) and non-attenders had a higher mortality (HR: 1.36, 95% CI 1.07 - 1.72). **Conclusion:** In this large UK sample, population-based screening for type 2 diabetes was not associated with a reduction in all-cause, cardiovascular or diabetes-related mortality over a median 9.6 years. Attendance was associated with a significantly lower mortality risk. **Figure 1** Cumulative incidence of death in the screening and no screening control groups in the ADDITION-Cambridge trial



Clinical Trial Registration Number: ISRCTN86769081

Supported by: Wellcome Trust, MRC, NHS R&D support, NIHR

## 184

### Glycaemic control and mortality at the intensive care unit: differences between patients with and without diabetes mellitus

M.K. Sechterberger<sup>1</sup>, R.J. Bosman<sup>2</sup>, H.M. Oudemans-van Straaten<sup>2</sup>, J.B.L. Hoekstra<sup>1</sup>, J.H. De Vries<sup>1</sup>

<sup>1</sup>Internal Medicine, Academic Medical Centre, <sup>2</sup>Intensive Care Medicine, Onze Lieve Vrouwe Gasthuis, Amsterdam, Netherlands.

**Background and aims:** Hyperglycaemia, hypoglycaemia and increased glucose variability in critically ill patients are independently associated with intensive care unit (ICU) mortality. These associations might be different in patients with and without pre-existing diabetes mellitus (DM). We investigated the extent to which three measures of glycaemic control over the duration of ICU admission, namely mean glucose concentration, glucose variability and the occurrence of hypoglycaemia, were associated with ICU mortality in patients with and without pre-existing DM.

**Materials and methods:** We performed a single-centre retrospective database cohort study including patients admitted from January 2004 to July 2011 in a 24-bed medical/surgical ICU in a teaching hospital in the Netherlands. Hyperglycaemia was treated using a computerized glucose regulation protocol. Diabetic status was defined by the use of anti-diabetic drugs at ICU admission. The non-DM and DM cohorts were subdivided into quintiles of increasing mean glucose and quartiles of glucose variability (measured as mean absolute glucose (MAG) change). Multivariable regression models were used to examine the independent association between ICU mortality and mean glucose, MAG change, and the occurrence of severe ( $\leq 2.2$  mmol/l) and mild ( $\leq 4.7$  mmol/l) hypoglycaemia.

**Results:** In total, 10,328 patients (8690 non-DM patients and 1638 DM patients) were eligible for analysis. Regarding mean glucose concentration, a U-shaped curved relation was observed in the non-DM cohort with significant increased ICU mortality in the lowest and highest mean glucose quintiles (OR 1.4 and 1.8 respectively,  $p < 0.001$ ). No clear pattern was found in the DM-cohort with no significant association between mean glucose and ICU mortality in logistic regression analysis ( $p$  for trend = 0.718). Glucose variability was only related with ICU mortality in the non DM-cohort, with significantly higher ICU mortality in the upper MAG change quartile (OR 1.62,  $P < 0.001$ ). Furthermore, in both cohorts the occurrence of severe hypoglycaemia ( $\leq 2.2$  mmol) was significantly associated with ICU mortality (adjusted

OR non-DM cohort: 2.5,  $p < 0.001$ ; DM cohort: 4.3,  $p = 0.001$ ). Mild hypoglycaemia ( $\leq 4.7$  mmol/L) was only significantly associated with ICU mortality in the non-DM cohort (adjusted OR 1.6,  $p < 0.001$ ).

**Conclusion:** Mild hypoglycaemia, high glucose variability and hyperglycaemia are related to ICU mortality in the non-DM, but not in the DM cohort, whereas severe hypoglycaemia was associated with ICU mortality in both. In the non-DM population, moderate targets seem therefore safest.

## 185

### Predictors of coronary artery disease in type 1 diabetes differ by level of glycaemic control

T.J. Orchard, R.G. Miller;

Epidemiology, University of Pittsburgh, Pittsburgh, USA.

**Background and aims:** Glycaemia has not been a consistent predictor of coronary artery disease (CAD) in T1D, however analyses are often based on a single HbA1c value. Thus, we examined further risk factors for CAD incidence in groups characterized by long-term HbA1c to explore whether these factors may partially explain why individuals with consistently good glycaemic control remain at elevated risk for CAD.

**Materials and methods:** Data are from 311 individuals in the Epidemiology of Diabetes Complications (EDC) study of childhood-onset ( $<17$  years old) T1D without baseline CAD and attendance at least 3 of the 7 possible examinations over the 18 year follow-up period (baseline mean age 27.0, T1D duration 18.4 yrs, 45% female). Incident CAD ( $n=82$ , 26%) was defined as a confirmed event (CAD death, myocardial infarction, revascularization/blockage  $\geq 50\%$ , ischemic ECG, or EDC physician-diagnosed angina) occurring prior to the 18-yr exam. The EDC cohort was divided into tertiles based on the mean of each participant's HbA1c percentile for each exam over the follow-up period. Individuals studied comprised those with a mean HbA1c percentile in the lowest 1/3 (Low A1c, 18-yr mean HbA1c=7.7% ) versus those in the highest 1/3 (High A1c, 18-yr mean HbA1c=10.0% ). Separate proportional hazards models for baseline risk factors were fit by HbA1c category.

**Results:** The High A1c group had significantly higher CAD incidence (31.9% versus 20.8% in Low A1c,  $p=0.03$ ). Univariately, standard lipid and blood pressure measures and estimated glomerular filtration rate (eGFR) predicted CAD in both A1c groups, however haemoglobin, haematocrit, red blood cell count, and white blood cell count predicted incident CAD in the Low A1c group only ( $p<0.05$  for all). After covariate adjustment, beyond diabetes duration, only hypertension remained a significant predictor of CAD in the High A1c group (HR=3.6,  $p=0.001$ ), while in the Low A1c group hypertension (HR=2.4,  $p=0.04$ ) and lower eGFR (per SD decrement HR=1.6,  $p=0.02$ ) were significant predictors of CAD. Indeed, there was a significant interaction between A1c and eGFR ( $p=0.006$ ), such that within the Low A1c group, individuals with low eGFR ( $<60$  ml/min/1.73m<sup>2</sup>) developed CAD more frequently than those without low eGFR (53% versus 17%,  $p=0.003$ ), while in the High A1c group, this difference was not as marked and 31% of individuals without low eGFR went on to develop CAD (versus 50% with low eGFR,  $p=0.29$ ).

**Conclusion:** These data suggest risk factor effects differ by glycaemic status and emphasize that, in the setting of lower HbA1c in T1D, renal function may be a particularly important factor in the development of CAD.

Supported by: NIDDK/NIH

## 186

### Diabetes management among adults under excellent control in the type 1 diabetes exchange clinic registry: How do they do it?

J.B. McGill<sup>1</sup>, V. Chen<sup>2</sup>, R.W. Beck<sup>3</sup>

<sup>1</sup>School of Medicine, Washington University in St. Louis, <sup>2</sup>Biostatistics, Jaeb Center for Health Research, Tampa, <sup>3</sup>Jaeb Center for Health Research, Tampa, USA.

**Background and aims:** Optimizing glycemic control in type 1 diabetes is important to minimize the risk of complications. Why are some patients able to achieve excellent control and others are not? We used the large T1D Exchange database to identify differences in patient characteristics and in diabetes management techniques by comparing patients  $\geq 18$  years old with excellent control versus those with poorer control.

**Materials and methods:** The T1D Exchange includes a network of 67 clinics with a registry of approximately 25,000 individuals with type 1 diabetes. Among participants  $\geq 18$  years old, those with HbA1c levels  $<6.5\%$  ( $N=889$ )

were compared with those with HbA1c levels  $\geq 8.5\%$  ( $N=2314$ ) using data obtained through a standardized data collection. Statistical comparisons were made between the two groups.

**Results:** Compared with the participants with HbA1c levels  $\geq 8.5\%$ , participants with HbA1c levels  $< 6.5\%$  were more often non-Hispanic whites, married, and working full time; had higher education and income levels; and more often had private insurance ( $P<0.001$  for each factor). With respect to diabetes management, participants with HbA1c levels  $< 6.5\%$  more often used an insulin pump, gave meal bolus before rather than after starting a meal, varied insulin: carb ratio for breakfast, lunch, and dinner; checked the blood glucose level  $\geq 6$  per day; and checked the blood glucose before giving a bolus, as seen in the table below ( $P<0.001$  for each factor). Daily total insulin/kg was lower in the group with better control ( $0.6\pm 0.3$  versus  $0.8\pm 0.4$  units/kg/day,  $P<0.001$ ).

	HbA1c <6.5%	HbA1c $\geq 8.5\%$
Non-Hispanic White	94%	81%
Education: Bachelor's or higher	63%	24%
Annual income $\geq \$75,000$	63%	36%
Married	58%	29%
Working full time	50%	33%
Private insurance	86%	70%
Using insulin pump	60%	48%
Gives insulin bolus prior to starting meal	67%	47%
Varied insulin:carbohydrate ratio at the 3 meals	37%	21%
Blood glucose measurements $\geq 6$ times/day	53%	18%
Always checks blood glucose before bolusing	52%	25%

**Conclusion:** Individuals with excellent glycemic control tend to be of higher socio-economic status than those with poorer control. They also tend to manage their diabetes differently with respect to insulin delivery modality, frequency of blood glucose monitoring, timing of meal boluses, use of insulin: carbohydrate ratios, and require lower insulin doses on average.

*Supported by: Leona M. & Harry B. Helmsley Charitable Trust Grant*

## OP 32 Insights in diabetic retinopathy

187

### Yield of referable retinopathy in the Scottish diabetic retinopathy screening programme

H.C. Looker<sup>1</sup>, D. Cromie<sup>2</sup>, Scottish Diabetic Retinopathy Screening Collaborative, Scottish Diabetes Research Network Epidemiology Group; <sup>1</sup>Medical Research Institute, University of Dundee, <sup>2</sup>Department of Public Health, NHS Lanarkshire, Glasgow, UK.

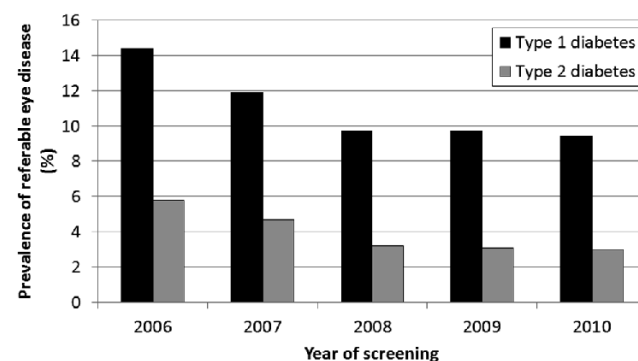
**Background and aims:** Screening for diabetic retinopathy is a vital part of diabetes care. Scotland launched a national diabetic retinopathy screening programme in 2006 with annual photography for all patients aged  $\geq 12$  years. We examined the yield of disease requiring referral to specialist eye care for the first 5 years of the programme.

**Materials and methods:** Retinal screening results were obtained from the national clinical diabetes database (SCI-DC) which contains data on  $\sim 99\%$  of people with diagnosed diabetes in Scotland. Those with severe retinal haemorrhages, venous beading or intra-retinal microvascular anomalies (referable retinopathy), new vessels (proliferative) and those with exudates or blot haemorrhages adjacent to the fovea (referable maculopathy) on centrally graded photographs are referred to specialist eye care.

**Results:** 21,295 people with type 1 diabetes and 169,072 people with type 2 diabetes underwent at least one successful retinal screening between 2006 and 2010 (median screens per person = 2). The first 2 years of screening had the highest yield of referable eye disease (6.9% and 5.4%) before stabilizing at 3.8% (standard error 0.05%). The prevalence of referable eye disease for type 1 diabetes was higher than for type 2 diabetes (see figure) in each year (Odds ratio for type 1 versus type 2 diabetes 3.5 (95% Confidence Interval 3.2–3.8) after adjustment for age and sex. The risk associated with type 1 diabetes was not uniform across all diabetes durations. For diabetes durations  $< 10$  years the OR for Type 1 diabetes vs. type 2 diabetes was 0.7 (0.5 – 0.9) whereas for durations  $\geq 10$  years the OR was 1.3 (1.2 – 1.4) (all OR adjusted for age and sex).

**Conclusion:** The annual national retinopathy screening programme in Scotland has yielded a substantial number of cases of referable disease leading to preventive action in the first five years of operation. This is especially the case for T1D for people with a diabetes duration  $\geq 10$  years. Further review of the yield will enable on-going cost-benefit analysis of the programme.

### Prevalence of referable diabetic eye disease at retinal screening



*Supported by: Wellcome Trust via Scottish Health Informatics Programme Grant (WT086113)*

188

### CACNB2 is a novel susceptibility gene for diabetic retinopathy

N. Vuori<sup>1</sup>, K. Hietala<sup>2,1</sup>, N. Sandholm<sup>1</sup>, C. Forsblom<sup>1,3</sup>, P. Summanen<sup>2</sup>, M. Lehto<sup>1</sup>, P.-H. Groop<sup>1,3</sup>

<sup>1</sup>Folkhälsan Research Center, <sup>2</sup>Department of Ophthalmology, Helsinki University Central Hospital, <sup>3</sup>Division of Nephrology, Department of Medicine, Helsinki University Central Hospital, Finland.

**Background and aims:** We have recently performed a linkage analysis on diabetic retinopathy, where we identified a region on chromosome 10 harbour-

ing an interesting candidate gene, *CACNB2*. The gene encodes the voltage-dependent L-type calcium channel subunit beta-2, which previously has been shown to associate with night-blindness in mice. A microsatellite (D10S548) was found in *CACNB2* with a LOD score of 2.73. The aim of this study was to confirm the linkage finding in a large scale case-control study of patients with type 1 diabetes.

**Materials and methods:** The patient population consisted of 1350 cases with severe diabetic retinopathy (SDR) and 1543 controls without SDR from the Finnish Diabetic Nephropathy (FinnDiane) Study. SDR was defined based on the presence of laser treatment and the controls were required to have at least 15 years duration of diabetes without SDR. The association study was performed as a lookup of our GWAS data originally collected for diabetic nephropathy. We selected genotyped SNPs within the gene and 10 kb upstreams and downstreams of the gene ( $n = 163$ ). Logistic regression with sex, age, and duration of diabetes as covariates was used for the analyses.

**Results:** 62 SNPs showed a  $p$ -value of  $< 0.05$  and 15 SNPs had a  $p$ -value of  $< 0.01$ . The SNP with the lowest  $p$ -value for severe retinopathy was rs4548524 with an OR of 0.819 (95% CI 0.671–0.990) and a  $p$ -value of 0.001. This SNP would not survive a robust correction for multiple testing, however, this particular SNP was closely located ( $< 5$  kb) to the linkage marker D10S548 with the highest LOD-score in the linkage analysis, thus supporting the importance of this locus. Nimblegen sequencing of an area of 219,738 bp on the gene that encompasses the linkage and association findings, is ongoing in an effort to identify potential gene variants associated with SDR. Preliminary data show two novel missense mutations (Arg-Cys, Ser-Leu) at the c-terminal half of the protein.

**Conclusion:** Our finding supports the hypothesis that variants in the gene *CACNB2* may confer genetic susceptibility to diabetic retinopathy in patients with type 1 diabetes.

*Supported by: Folkhälsan Research Foundation, Stockmann Foundation*

## 189

### Activated microglial cells promote vasoregression

S. Busch<sup>1</sup>, L. Wu<sup>1</sup>, N. Gretz<sup>2</sup>, S. Hoffmann<sup>2</sup>, H.-P. Hammes<sup>1</sup>

<sup>1</sup>5th Medical Department, <sup>2</sup>Medical Research Center, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany.

**Background and aims:** Neurodegeneration, activation of glial cells and vasoregression are hallmarks of early diabetic retinopathy. A similar pathomechanism is found in the euglycemic, neurodegenerative PKD rat. Microglial activation has been shown by upregulation of CD74, the invariant chain of MHCII. The increase was predominantly localized in the deep capillary layer of the PKD model, the layer which is commonly concerned by retinopathy at first. Consequently, microglial cells could mediate vasoregression. To determine the extent of vasoregression caused by microglial activation, number of acellular capillaries in the PKD model was evaluated after depletion of microglial cells.

**Materials and methods:** Transgenic rats TGR(CMV-PKD2<sub>(1/703)</sub>,HA) of one and two months were treated with intravitreal injection of 3  $\mu$ l Clodronate-coated liposomes. Animals were sacrificed after one week and eyes were enucleated. Untreated animals were used as controls. After fixation with formalin, retinae were taken out and incubated with primary antibodies against CD11b (AbD Serotec MCA275G) and CD74 (Santa Cruz sc20082). Swine-anti-rabbit Tritic (Dako R0156) and donkey-anti-goat AlexaFluor488 (Invitrogen A11055) were used as secondary antibodies. Confocal laser scanning microscopy was used to evaluate the number of positive staining for CD11b and CD74 in different retinal layers. Quantification of acellular capillaries occurred in retinal digest preparations. Formalin-fixated eyes were treated for 1.5h with pepsin, followed by 15–30min trypsin digestion. The remaining vessel network was stained with PAS and hematoxylin. Acellular capillaries were counted by using an integration ocular.

**Results:** Number of CD11b positive cells were increased by 27% ( $p < 0.05$ ) in the superficial and 400% ( $p < 0.01$ ) in the deep vessel network of PKD rats compared with control animals. Expression of CD74 in the superficial vessel network was threefold higher ( $p < 0.05$ ). In comparison with control rats, which don't express CD74 in the deep vessel network, 83% of all CD11b positive cells in the deep vessel network of PKD rats were also positive for CD74. Treatment with Clodronate reduced number of positive CD11b cells in the superficial layer by 27% ( $p < 0.05$ ). Superficial CD74 expression increased eightfold ( $p < 0.01$ ), whereas CD74 expression in the deep vessel network was reduced by 50% ( $p < 0.05$ ). Number of acellular capillaries in the superficial vessel network decreased after treatment of 1 month old animals by 14% (n.s.). The reduction in the deep vessel network was 30% ( $p < 0.01$ ). In

2 months old animals, number of superficial acellular capillaries increased by 5% (n.s.), whereas number of acellular capillaries in the deep vessel layer declined by 28% ( $p < 0.05$ ).

**Conclusion:** Number of acellular capillaries, caused by transgenic neurodegeneration, can be reduced by depletion of microglial cells. Therefore, activation of the innate immunity system, especially microglial cells, promotes vasoregression. Consequently, microglial cells and prevention of their activation could be a target to treat vasoregression.

*Supported by: DDG, DFG (GRK880/3)*

## 190

### High glucose induces mitochondrial dysfunction in retinal Muller cells: implications for diabetic retinopathy

T. Tien, T. Muto, S. Roy;  
Boston University, USA.

**Background and aims:** Muller cell loss has been associated with the development of early stage diabetic retinopathy, a leading cause of blindness in the working age population. In the retina, Muller cells are intimately associated with the vasculature and the neurons and play a key role in the maintenance of overall retinal homeostasis and function. Hyperglycemia-induced oxidative stress has been implicated in promoting Muller cell apoptosis in diabetic retinopathy, and recent studies indicate that changes in mitochondrial morphology increase oxidative stress and trigger apoptosis. However, it is unclear whether high glucose induces mitochondrial dysfunction and promotes apoptosis in retinal Muller cells. In this study, we determined whether high glucose promotes apoptosis in rat retinal Muller cells (rMC-1) by inducing biochemical and morphological changes in mitochondria.

**Materials and methods:** To determine if high glucose (HG) induces mitochondrial morphology changes, rMC-1 were grown in normal (N, 5mM glucose) or HG (30mM) medium for 6 days. Cells were stained with MitoTracker Red, and digital images were captured through live cell imaging under confocal microscopy. Images were analyzed for mitochondrial morphology changes based on Form Factor (FF) and Aspect Ratio (AR). Temporal effects of HG on mitochondrial morphology were examined at 15, 30, 45, 60, 90, and 120 minutes by analyzing FF and AR changes. Altered mitochondrial metabolic function under HG was assessed by measuring oxygen consumption rate (OCR) using XF24 bioenergetic assay. In parallel, TUNEL assay was performed to determine cells undergoing apoptosis, and cytochrome c levels were assessed using Western blot analysis.

**Results:** Cells grown in HG medium exhibited significantly increased mitochondrial fragmentation compared to those grown in N medium (FF =  $1.7 \pm 0.1$  vs  $2.3 \pm 0.1$ ; AR =  $2.1 \pm 0.1$  vs  $2.5 \pm 0.2$ ;  $p = 0.002$  and  $0.03$ , respectively). Time course studies indicate that HG-induced mitochondrial fragmentation follows a bimodal pattern in which initial fragmentation is followed by a recovery period; however, after 48h of HG exposure, permanent mitochondrial fragmentation was observed for the rest of the study. OCR was significantly reduced in rMC-1 grown in HG medium compared to those grown in N medium (76%  $\pm$  13% of control). The number of TUNEL positive cells was significantly increased in cells grown in HG medium compared to those grown in N medium (183%  $\pm$  35% of control,  $p = 0.002$ ) with concomitant increase in cytochrome c levels (247%  $\pm$  94% of control,  $p = 0.03$ ), as determined from WB analysis.

**Conclusion:** Findings from this study indicate that HG promotes mitochondrial dysfunction in retinal Muller cells by inducing mitochondrial morphology changes with concomitant increase in cytochrome c and apoptosis. HG-induced mitochondrial morphology changes and subsequent mitochondrial dysfunction may play a key role in retinal Muller cell loss associated with diabetic retinopathy.

*Supported by: NIH, NEI EY018218*

## 191

### Neurophysiological alterations in a mouse model of proliferative retinopathy

P. Villacampa<sup>1</sup>, V. Haurigot<sup>1</sup>, S. Motas<sup>1</sup>, A. Ribera<sup>1</sup>, L. Ramirez<sup>2</sup>, P. de la Villa<sup>2</sup>, F. Bosch<sup>1</sup>

<sup>1</sup>Universitat Autònoma de Barcelona, Bellaterra, <sup>2</sup>Universidad de Alcalá, Alcalá de Henares, Spain.

**Background and aims:** Diabetic retinopathy is the leading cause of loss of visual acuity and blindness in adulthood and constitutes an unmet medical



need. Most diabetic rodents do not develop a complete phenotype of proliferative retinopathy, but only mild vascular alterations. In contrast, transgenic mice overexpressing Insulin-like Growth Factor (Tg-IGF-I) in the retina have retinal alterations characteristic of non-proliferative retinopathy that, with age, develop to proliferative disease, with neovascularization and retinal detachment. Then, vascular alterations evolve in a progressive manner through the animal's life, similar to those of the human disease. The aim of this work was to determine whether neuronal and glial function were also affected in these animals, as observed in human diabetic patients.

**Materials and methods:** Electroretinographic (ERG) responses were recorded in dark-adapted mice (3, 6, 7.5 and 9 months-old TgIGF-I and age-matched wild-types (Wt), with intensities that ranged from -4 to 2 log cd•s•m<sup>-2</sup>. Immunohistochemistry for neuronal and glial markers was performed in paraffin-embedded retinal sections, and neuronal populations were quantified by morphometric analysis. The expression of genes key to glial metabolism were studied by qRT-PCR. Glutamate and glutathione quantity and glutamine synthetase activity were assessed spectrophotometrically. Other markers of oxidative stress were analyzed by using Western blot and qPCR.

**Results:** No alterations were observed in the ERG of 3 months-old TgIGF-I mice, in neither scotopic nor photopic conditions. At 6 months of age, abnormal responses were detected in some but not all TgIGF-I, but it was not until 7.5 months of age that differences became statistically significant. At this point, all transgenic mice showed more than 50% reduction in amplitudes of both a and b waves in all range of intensities. Furthermore, all mice aged 9 months were completely blind. By histological analysis, decreased populations of bipolar, amacrine and ganglion neurons were found at 7.5 months of age. Moreover, rod photoreceptors showed decreased outer segment length. Gliosis and microgliosis were detected in transgenic mice at early ages. Transgenic mice showed alterations in the glutamate metabolism, signs of oxidative stress and impaired potassium buffering. Increased production of pro-inflammatory cytokines such as Tumour Necrosis Factor alpha (TNF-α) and Monocyte Chemoattractant Protein-1 (MCP-1) were also observed at this age.

**Conclusion:** TgIGF-I mice presented a progressive decline in retinal neuronal function and reduced cellular populations. Loss of vision correlated in time with overt retinal neovascularization, vascular leakage and was preceded by glial and microglial activation. Glial dysfunction may underlie neuronal damage in transgenic retinas, which could be exacerbated by increased production of pro-inflammatory cytokines. Most of these glial alterations have also been reported in human diabetic retinas, making TgIGF-I a suitable model for the study of new therapeutic approaches for diabetic retinopathy. Supported by: Plan Nacional I+D+I (SAF2008-00962, SAF2011-24698), Spain

(RBZ+laser) RBZ injections in the 2<sup>nd</sup> year, with a mean BCVA change of +7.9 (RBZ) and +6.7 (RBZ+laser) letters at M24. The mean CRT change at M12 (RBZ: -127.8μm and RBZ+laser: -139.7μm) was maintained at M24 (RBZ: -140.6μm and RBZ+laser: -133.0μm). In patients treated with only laser in the core phase, mean BCVA gain of +2.3 letters at M12 further improved to +5.4 letters at M24 with an average of 4.1 RBZ injections in the 2<sup>nd</sup> year; mean CRT decreased further from -63.3μm (M12) to -126.6μm (M24). Mean change in VF (Day1-M24) for RBZ/RBZ+laser/laser was +10.8/+10.5/+4.6 points (near activity), +5.1/+7.0/+2.8 points (distance activity) and +5.6/+5.8/+4.3 points (composite score). 3-year (Day1-M36) results of safety and efficacy of RBZ will also be presented.

**Conclusion:** The AEs reported over 2 years were consistent with the published safety profile of RBZ in DME. In the 2<sup>nd</sup> year, an average of 3.8 RBZ injections over the three core treatment groups was sufficient to maintain (RBZ/RBZ+laser) or improve (laser) BCVA, and CRT outcomes in the RESTORE extension study. RBZ treatment was associated with VF improvements in all treatment groups over 2 years. The outcome of the RESTORE extension study provides additional evidence for the long-term safety and efficacy of RBZ in DME.

*Clinical Trial Registration Number: NCT00906464*

*Supported by: Novartis Pharma AG, Switzerland*

## 192

### Long-term safety and efficacy of ranibizumab 0.5 mg in patients with diabetic macular oedema of the RESTORE extension study

G.E. Lang, on behalf of the RESTORE Extension Group;  
Ophthalmology, University Eye Hospital Ulm, Germany.

**Background and aims:** Diabetic macular edema (DME) is the leading cause of blindness in diabetic patients (pts). The RESTORE core study was the first study to demonstrate that ranibizumab monotherapy (RBZ) or combined with laser (RBZ+laser) achieved superior visual acuity gain vs laser monotherapy in patients with visual impairment due to DME. The RESTORE extension study provides long-term evidence for safety and efficacy of RBZ in DME.

**Materials and methods:** 24-month (M), open-label, multicenter, Phase-IIIb, extension study. Among the 303 pts who completed RESTORE core study (Day1-M12) 240 pts (79%) entered the extension study (M12-M36); all pts (RBZ/RBZ+laser/laser) were eligible to receive RBZ PRN according to pre-specified stability-based best-corrected visual acuity (BCVA) and DME progression re-treatment criteria, and laser PRN according to Early Treatment Diabetic Retinopathy Study guidelines. Interim analysis at M24 (12M core phase + 12M extension phase[Day1-M24]) and full analysis at M36 (12M core phase + 24M extension phase[Day1-M36]) of the incidence of adverse events (AEs), treatment exposure, changes in BCVA, patient-reported visual functioning (VF, assessed by Visual Functioning Questionnaire [VFQ-25]) and central retinal subfield thickness (CRT) are presented. Last observation carried forward approach was used for BCVA, VF and CRT analysis.

**Results:** 220 pts (92%) completed the M24 visit. Neither new AEs nor new safety risks were revealed in the 2-year analysis. There were no cases of endophthalmitis. There were 4 deaths between M12-M24 (none related to study drug/procedure). The mean BCVA gain of +7.9 (RBZ) and +7.1 (RBZ+laser) letters at M12 was maintained with an average of 3.9 (RBZ) and 3.5

## OP 33 Devices, algorithms and their application

193

### Short-term glucose prediction algorithm reduces hypoglycaemia among virtual patients

A. Roy, B. Keenan, G. Spital, B. Clark, B. Grosman, J.J. Mastrototaro, F.R. Kaufman;

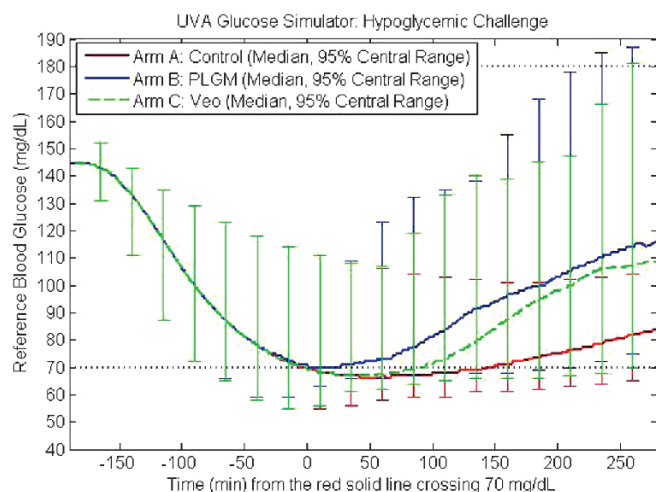
Medtronic Diabetes, Northridge, USA.

**Background and aims:** The Paradigm® Veo™ pump with a threshold low glucose suspend (LGS) interrupts basal infusion for a maximum of 2 hours once sensor glucose (CGM) values drop below a user-defined threshold. Clinical studies have shown that LGS minimizes time spent in hypoglycemia. However, a preferable approach might be to suspend insulin delivery 20–30 min in advance of a hypoglycemic event. This could be achieved by using a predictive low glucose management (PLGM) algorithm to predict future hypoglycemia, suspend basal insulin 30 minutes prior to the event, and then resume delivery based on predicted glucose levels. An *in silico* study with hypoglycemic challenges was done to compare the efficacy of LGS and PLGM in reducing the frequency and severity of hypoglycemic events.

**Methods:** Hypoglycemic challenges were simulated using the FDA-accepted University of Virginia glucose simulator with 20 virtual patients. The study consisted of three arms: Arm A - control (no pump suspension); Arm B - hypoglycemia mitigation via PLGM; Arm C - hypoglycemia mitigation via LGS. In order to induce hypoglycemia, the virtual patients received a manual bolus after the commencement of study. The bolus amount was patient specific (based on insulin sensitivity) and was chosen such that the blood glucose nadir was between 50 and 70 mg/dL. For both algorithms, the pump suspension threshold was set at 70 mg/dL. For PLGM, the 30 min glucose prediction was performed by a time-series forecasting approach.

**Results:** Figure below illustrates the time-series plot of reference glucose values (median, 2.5<sup>th</sup>–97.5<sup>th</sup> percentile range) of all arms. The data revealed that PLGM reduced hypoglycemia (<70 mg/dL) by 26.7% compared to no insulin suspension, as opposed to a 5.3% reduction in hypoglycemia with use of LGS. The mean duration of hypoglycemia (time spent <70 mg/dL) with PLGM was significantly less than with LGS (58 min vs 101 min, respectively,  $p < 0.001$ ). With PLGM, the mean ( $\pm$  SD) time gain in forecasting hypoglycemic events was 30.6 ( $\pm$  12.8) min.

**Conclusion:** *In silico* modeling has demonstrated that predictive algorithms may further reduce the severity of hypoglycemia beyond that already established for algorithms that use a threshold-based suspension.



194

### Time in hypoglycaemia in patients with type 1 diabetes is dramatically reduced when insulin infusion is driven by two closed-loop algorithms in a randomised clinical trial

E.M. Renard<sup>1</sup>, J.H. DeVries<sup>2</sup>, R. Hovorka<sup>3</sup>, W. Doll<sup>4</sup>, L. Heinemann<sup>5</sup>, C. Cobelli<sup>6</sup>, L. Magni<sup>7</sup>, A. Farret<sup>1</sup>, Y.M. Luijck<sup>2</sup>, L. Leelarathna<sup>3</sup>, J.K. Mader<sup>4</sup>, C. Benesch<sup>5</sup>, D. Bruttomesso<sup>6</sup>, F. Di Palma<sup>7</sup>, M. Nodale<sup>3</sup>;

<sup>1</sup>Department of Endocrinology, Diabetes, Nutrition, Montpellier University Hospital, France, <sup>2</sup>Department of Internal Medicine, Academic Medical Center at the University of Amsterdam, Netherlands, <sup>3</sup>Institute of Metabolic Science, University of Cambridge, UK, <sup>4</sup>Department of Internal Medicine, Medical University of Graz, Austria, <sup>5</sup>Profil Institute, Neuss, Germany, <sup>6</sup>University of Padova, Italy, <sup>7</sup>University of Pavia, Italy.

**Background and aims:** Closed loop algorithms built for model predictive control (MPC) aim to keep blood glucose close to normal in patients with Type 1 diabetes by the tuning of insulin delivery based on continuous glucose monitoring (CGM) and prediction of glucose levels.

**Materials and methods:** Blood glucose was controlled for 23 h in 47 patients in six centers by one of two MPC algorithms developed either at the University of Pavia with a Safety Supervision Module from UVA & UCSB (iAP) or at Cambridge University (CAM), or by the patients themselves in Open Loop (OL) mode, in a randomized three-way cross-over design during three clinical research center admissions including three meals and an exercise bout. CGM data were provided by the Dexcom Seven Plus (Dexcom Inc, San Diego, CA, USA) and insulin was administered by Omnipod Insulin Pump (Insulet Corp, Bedford, MA, USA), either automatically with the Artificial Pancreas System (UCSB/Sansum, CA, USA) (three centers) or manually (three centers). For glucose reference measurements the YSI glucose analyzer was used. Intention-to-treat (ITT) and per-protocol (PP) analyses were done using a general linear model for repeated measures. For the PP analysis, 0.4% (OL), 13% (iAP) and 17% (CAM) of time was discarded because of CGM or insulin pump malfunction or operator mistakes.

**Results:** Percentage of time spent in euglycaemia (3.9–8, postmeal 10 mmol/L) was similar in closed and open loop modes, both in ITT and PP analyses: 62.6 and 62.8 for OL, 59.2 and 59.3 for iAP, 58.3 and 59.6 for CAM. While mean glucose level (mmol/L) was significantly lower in open loop mode, both in ITT and PP analyses: 7.19 and 7.18 for OL, 8.15 and 8.27 for iAP, 8.26 and 8.15 for CAM (overall  $P=0.001$ ), % time spent in hypoglycaemia (<3.9 mmol/L) was almost threefold reduced during closed loop, both in ITT and PP analyses: 6.4 and 6.3 for OL, 2.1 and 0.9 for iAP, 2.0 and 0.0 for CAM (overall  $P=0.001$ ) with less % time  $\leq 2.8$  mmol/L in ITT and PP analyses (overall  $P=0.038$  and 0.017). There were no significant differences in any of these outcomes between the two closed loop algorithms. The safety of closed-loop mode was further supported by the absence of harmful glucose deviations in case of pump or CGM failure. The feasibility of automated management of the closed-loop systems was documented in the 3 investigation centers that tested it.

**Conclusion:** From the largest and first head-to-head closed loop study performed so far, we conclude that both CAM and iAP MPC algorithms allow safer control of blood glucose than patient self management, even during meal and exercise challenges, with less hypoglycaemia at the expense of a higher mean glucose. In future trials, tuning of the algorithms will aim at increased % time in target and lower mean glucose level while keeping the reduction of hypoglycaemia.

Clinical Trial Registration Number: ISRCTN62034905

Supported by: European Union FP7 grant 247138

195

### Efficacy and safety of reduced prandial boluses during closed-loop insulin delivery in adolescents with type 1 diabetes

D. Elleri<sup>1</sup>, J.M. Allen<sup>1</sup>, M. Biagioni<sup>1</sup>, K. Kumareswaran<sup>1</sup>, L. Leelarathna<sup>1</sup>, K. Caldwell<sup>1</sup>, M. Nodale<sup>1</sup>, M.E. Wilinska<sup>1</sup>, A. Haidar<sup>1</sup>, P. Calhoun<sup>2</sup>, C. Kollman<sup>3</sup>, M.A. Umpleby<sup>3</sup>, C.L. Acerini<sup>1</sup>, D.B. Dunger<sup>1</sup>, R. Hovorka<sup>1</sup>;

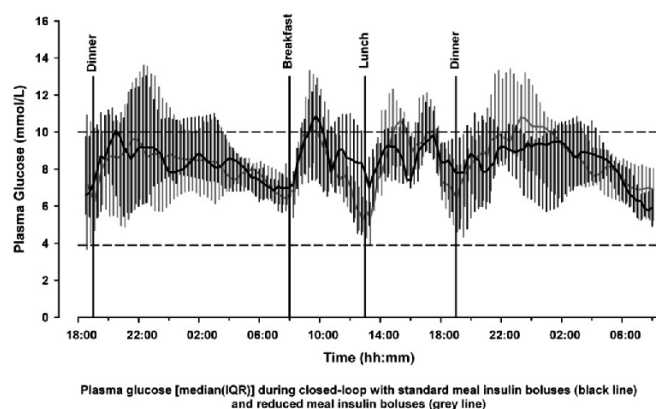
<sup>1</sup>University of Cambridge, UK, <sup>2</sup>Jaeb Center for Health Research, Tampa, USA, <sup>3</sup>Postgraduate Medical School, University of Surrey, Guildford, UK.

**Background and aims:** Closed-loop (CL) insulin delivery may be particularly efficacious when coupled with manual prandial insulin boluses. It is currently unknown if reducing prandial insulin boluses provides similar level of control whilst limiting post-prandial hyperinsulinemia and reducing risk of post-meal hypoglycaemia.

**Materials and methods:** Eight adolescents with type 1 diabetes [M 3; age  $15.9 \pm 1.5$  yrs; A1C  $8.9 \pm 1.6\%$ ; mean  $\pm$  SD; total daily dose  $0.9 (0.7, 1.1)$  IU/kg/d; median (IQR)] were studied over 36 hours (two nights and daytime) on two occasions. In random order, subjects were treated by CL with either standard insulin boluses calculated using subjects' pump bolus calculator or CL with boluses reduced by 25%. Boluses were given before main meals [(50–80 g carbohydrates (CHO)) but not with snacks (15–30 g CHO). Between-meal insulin delivery was adjusted every 15 min as per advice of a model-predictive-control algorithm informed by a real-time continuous glucose monitor. Subjects undertook moderate-intensity exercise on a stationary bicycle for 20 min in morning and afternoon. Stable-label tracer approach using intravenous [ $6,6\text{-}^2\text{H}_2$ ]glucose was employed to estimate total glucose appearance ( $R_{a\_total}$ ) and glucose disposal ( $R_d$ ).

**Results:** Overall insulin delivery was lower with reduced prandial boluses [ $61.9 (55.2, 75.0)$  vs  $72.5 (63.6, 80.3)$  IU,  $p=0.01$ ] and resulted in lower mean plasma insulin concentration [ $186 (171, 260)$  vs  $252 (198, 336)$  pmol/l,  $p=0.002$ ] but without change in plasma insulin clearance [ $16.6 (9.2, 20.9)$  vs  $15.7 (8.3, 17.5)$  ml/kg/min,  $p=0.16$ ]. Mean plasma glucose was identical ( $8.4 \pm 0.9$  mmol/l,  $p=0.97$ ). Time when plasma glucose was in target range  $3.9\text{--}10$  mmol/l was comparable [ $74 (66, 84)\%$  vs  $80 (65, 96)\%$ ,  $p=0.87$ ] and so was time above  $10$  mmol/l [ $21.8 (16.3, 33.5)\%$  vs  $18.0 (4.1, 34.2)\%$ ,  $p=0.87$ ] and time below  $3.9$  mmol/l [ $0 (0, 1.5)\%$  vs  $0 (0, 1.8)\%$ ,  $p=0.88$ ]. Hypoglycaemia occurred once 1.5 h after a meal during CL with standard bolus.  $R_{a\_total}$  was similar [ $26.3 (21.9, 28.0)$  vs  $25.4 (21.0, 29.2)$   $\mu\text{mol/kg/min}$ ,  $p=0.19$ ] as well as  $R_d$  [ $25.8 (21.0, 26.9)$  vs  $25.2 (21.2, 28.8)$   $\mu\text{mol/kg/min}$ ,  $p=0.46$ ].

**Conclusion:** Closed-loop insulin delivery coped well with 25% reduction of prandial boluses achieving glucose control and glucose turnover not different from that achieved by closed-loop with standard boluses but at reduced insulin levels. This suggests that large insulin boluses may lead to acute insulin resistance. Closed-loop with reduced boluses may be favourable in adolescents with type 1 diabetes.



Supported by: NIDDK R01DK085621

## 196

### Postprandial glucose control by catheter based pumps versus patch pumps and the influence of wear time

Y.M. Luijck<sup>1</sup>, A. Avogaro<sup>2</sup>, C. Benesch<sup>3</sup>, D. Bruttomesso<sup>2</sup>, L. Heinemann<sup>3</sup>, J. Place<sup>4</sup>, E. Renard<sup>4</sup>, R. Scotton<sup>2</sup>, J.H. DeVries<sup>1</sup>, AP@home consortium;

<sup>1</sup>Internal Medicine, Academic Medical Centre, Amsterdam, Netherlands, <sup>2</sup>Department of Clinical and Experimental Medicine, University of Padova, Italy, <sup>3</sup>Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany, <sup>4</sup>Internal Medicine, Montpellier University Hospital, France.

**Background and aims:** Most closed-loop systems rely on the subcutaneous administration of insulin by means of conventional insulin pumps with a catheter. Recently patch pumps have been developed to be worn directly on the body with an internal catheter of only a few centimetres between the insulin reservoir and infusion site. Both catheter length and wear time are thought to influence insulin administration. The aim of this study was to measure blood glucose profiles after bolus insulin administration by either a patch pump (PP) or a catheter based pump (CP) and the effect of catheter wear time on these profiles.

**Materials and methods:** 18 patients aged 18 or above with type 1 diabetes were recruited in 4 clinical centres of our consortium. Patients came in for two blocks of visits: one block of two visits while wearing the OmniPod In-

sulin Pump (PP visit 1 and 2) and one block of two visits while wearing the Medtronic Paradigm Pump (CP visit 1 and 2) with a 60 cm Teflon catheter. The visit blocks were in random order, using a crossover design. The first and second visits within a block were 48 hours apart to allow assessment of the effect of wear time. Patients were served an identical breakfast on all study visits and received an identical insulin bolus of at least 6 IU to cover the meal. Venous blood samples were drawn for glucose determination from one hour before breakfast until 4.5 hours afterwards. Glucose outcomes were analysed using a General Linear Model with Repeated Measures analysis (GLM-RM). If the GLM-RM detected significant overall differences, a paired t-test was performed between glucose profiles on day 1 and day 3 of wear of both pumps (intra-pump changes), and between day 1 of both pumps and day 3 of both pumps (inter-pump changes).

**Results:** Maximum blood glucose excursion (mean (SD)) was significantly higher on visit day 1;  $129.1$  mg/dL ( $38.3$ ) for PP visit 1 versus  $97.8$  ( $41.5$ ) for PP visit 2 ( $P=0.006$ ) and  $127.0$  ( $47.0$ ) for CP visit 1 versus  $96.6$  ( $31.2$ ) for CP visit 2 ( $P=0.002$ ). There was no inter-pump difference on day 1 ( $P=0.842$ ) or day 3 ( $P=0.866$ ). Average postprandial blood glucose was significantly higher on study days 1 ( $P=0.012$ );  $170.0$  mg/dL ( $47.3$ ) for PP visit 1 versus  $141.8$  ( $48.1$ ) for PP visit 2 ( $P=0.016$ ) and  $163.7$  ( $45.1$ ) for CP visit 1 versus  $141.9$  ( $37.7$ ) for CP visit 2 ( $P=0.052$ ). There was no inter-pump difference for day 1 ( $P=0.641$ ) or day 3 ( $P=0.995$ ). Time to peak glucose did not differ between study visits ( $P=0.317$ ).

**Conclusion:** Maximum blood glucose excursion and average glucose were both lower on day 3 of pump use. There were no differences between patch pumps and conventional pumps. These findings suggest that wear time plays an important role in subcutaneous insulin administration.

Supported by: The European Commission under the FP7 program, grant number 247138

## 197

### Continuous subcutaneous insulin infusion is superior to subcutaneous insulin injection in the desensitisation therapy of insulin allergic diabetic patients

T. Yuan, N.S. Li, W.G. Zhao;

Department of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Beijing, China.

**Background and aims:** Insulin allergy is still a major problem in the management of diabetes, although its incidence has dramatically decreased since the introduction of human insulin preparations. Immediate type IgE-mediated reactions (type I) are the most common presentation of insulin allergy. We wanted to compare two different methods in the insulin desensitisation therapy.

**Materials and methods:** We retrospectively analysed 13 cases of diabetic patients who all presented with local skin reactions after subcutaneous injection of different kinds of insulin preparations. According to the local skin reaction including the onset and disappearance time and the area of erythema, wheal, induration or pseudopodia, we selected one of the lowest reaction insulin preparations as the desensitisation insulin. Then we divided the patients into two groups accepting desensitisation therapy (continuous subcutaneous insulin infusion (CSII),  $n=5$ ; or multiple subcutaneous insulin injection,  $n=8$ ) depending on the willingness of the patients. We followed the pilot procedure in the previous treatment of insulin allergic patients in these two groups respectively. In the CSII group, the short acting insulin preparation was diluted 10 times (from U100 to U10 using saline). We used Minimed pump in all these patients. The basal rate of diluted insulin began with  $0.1$  u/h (actually was  $0.01$  u/h of insulin), then increased the basal infusion rate with  $0.1$  u per hour (actually increased with  $0.01$  u insulin per hour) in the first day of desensitisation. At the end of the first day, the basal rate would increase to  $2.4$  u/h (actually was  $0.24$  u/h). If the patient was free of any symptom of allergy, we would start the basal rate from  $0.25$  u/h in the second day (using the U100 insulin without dilution), increased the rate by  $0.05$  u per hour, till  $1.4$  u/h at the end of the second day. In the third day of desensitization, we kept the basal rate as  $1.0$  u/h or decreased to  $0.5$  u/h at night to avoid hypoglycemia in older patient, added the bolus insulin 4–6u before meal with the insulin pump. In the multiple subcutaneous insulin injection desensitisation group, The initial dose was  $0.00001$  units, with subsequent doses progressively increasing 10-fold up to 1 unit with the interval of injection was 1 hour, then 2, 4, 8, 12, 16, and 20 units. In case of local allergic reactions, the last dose was repeated until no reaction occurs and then the dose increases were continued.

**Results:** The benefit effects of CSII in the desensitisation therapy are shortening of desensitisation period (from 6.9 days to 3 days), increasing the success



rate of therapy (from 87.5% to 100%), convenient for the doctors and the nurses to operate, decreasing the discomfort of the patients and increasing the compliance of the patients.

**Conclusion:** In our observation, CSII is superior to multiple subcutaneous insulin injection by syringe in the desensitisation therapy of insulin allergic patients.

## 198

### Accuracy of a new laser technology device for the non invasive measurement of glucose in man

M. Scavini<sup>1</sup>, C. Molinari<sup>2</sup>, G.A. Di Terlizzi<sup>2</sup>, A.M. Bolla<sup>2</sup>, F. Perticone<sup>2</sup>, F. Ceriotti<sup>3</sup>, P. Trombetta<sup>4</sup>, E. Bosi<sup>1</sup>;

<sup>1</sup>Diabetes Research Institute, San Raffaele Scientific Institute, Milan, <sup>2</sup>San Raffaele Scientific Institute, Milan, <sup>3</sup>Laboraf Diagnostica e Ricerca San Raffaele, Milan, <sup>4</sup>Gulya S.r.l., Como, Italy.

**Background and aims:** A truly non invasive glucose monitoring would represent an outstanding advancement for the management of diabetes. Aim of this study was to test the accuracy of Glycolaser<sup>®</sup>, a new device for the non invasive glucose measurement based on an innovative laser technology.

**Materials and methods:** We studied male and female healthy volunteers, patients with type 1 or type 2 diabetes, patients with hypoglycemia, aged 18 to 75 yrs. Participants were studied in one occasion, either fasting or in post prandial conditions. Glycolaser<sup>®</sup> measurements were compared with plasma glucose measured with the hexokinase method on a venous blood sample drawn simultaneously. Agreement of glucose measurements with the two methods was analyzed using: a) the bias plot (ISO limits: within  $\pm 15$  mg/dl for glucose values  $< 75$  mg/dl and within  $\pm 20\%$  for glucose values  $\geq 75$  mg/dl); b) the Clarke Error Grid; and c) the Parkes Error Grid.

**Results:** We studied 171 adults aged  $47.2 \pm 16.9$  yrs, 40.4% females: 31 controls, 136 patients with type or type 2 diabetes and 4 patients with hypoglycemia. Participants were studied either fasting (n=137) or in post prandial (n=34) conditions. Forty-nine percent of Glycolaser<sup>®</sup> measurements were within the ISO limits (7.7 % for glucose levels  $< 75$  mg/dl and 52.5 % for glucose levels  $\geq 75$  mg/dl). The distribution of Glycolaser<sup>®</sup> measurements in the regions of the Clarke Error Grid was A 47.4 %; B 41.5 %; C 1.7%; D 8.2 %; E 1.2 %, with 88.9% of Glycolaser<sup>®</sup> measurements in clinically acceptable regions (A+B). The distribution of Glycolaser<sup>®</sup> measurements in the regions of the Parkes Error Grid was A 56.7 %; B 33.3 %; C 8.2 %; D 8.21.8 %; E 0 %, with 90.0 % of Glycolaser<sup>®</sup> measurements in clinically acceptable regions (A+B). Sex, age, fasting or postprandial condition did not influence the accuracy of Glycolaser<sup>®</sup> measurements.

**Conclusion:** Although the accuracy of the present device is not yet fulfilling the ISO standards, the overall performance of the non invasive Glycolaser<sup>®</sup> is not far from that of the best invasive blood glucose meters currently on the market. The improvement of the performances of the Glycolaser<sup>®</sup> prototype is expected to allow the accurate non invasive measurement of glucose in patients with diabetes in the near future.

*Supported by: Gulya S.r.l.*

## OP 34 Profiling glucose and clinical trials

### 199

#### Evaluation of tracking of glycaemic variability in well controlled type 2 diabetes patients: 4 years results from the ORIGIN CGM substudy

C. Koehler<sup>1</sup>, F. Schaper<sup>2</sup>, J. Steiner<sup>1</sup>, R. Staudte<sup>1</sup>, W. Landgraf<sup>3</sup>, M. Hanefeld<sup>2</sup>; <sup>1</sup>Medical Consulting, GWT-TUD GmbH, Dresden, <sup>2</sup>Center for Clinical Studies, GWT-TUD GmbH, Dresden, <sup>3</sup>Sanofi-aventis Deutschland GmbH, Frankfurt, Germany.

**Background and aims:** Continuous glucose monitoring (CGM) is an appropriate method to evaluate glycaemic variability (GV). However, there is limited information available on the tracking of CGM of an individual glucose profile. The main objective of this study was to determine whether an individual CGM profile is repeatable with stable glucose control after 4 years. **Materials and methods:** 44 T2D patients from the ORIGIN trial with a stable drug treatment for diabetes and a well-controlled HbA1c ( $< 6.2\%$ ) during follow up were included. CGM system (Medtronic) was used to measure 72h of interstitial glucose (IG) at baseline and after 4 years. Area under the curve (AUC) over 24h of the 2<sup>nd</sup> and 3<sup>rd</sup> day, 2h after testmeal (TM) at the 2<sup>nd</sup> day, peak of IG during TM, standard deviation of mean IG (SD) and mean average glucose excursions (MAGE) were calculated. In addition, anthropological data, fasting plasma glucose, HbA1c and BMI were measured.

**Results:** The correlation between baseline and 4 year follow-up (FU) of determination of MAGE and SD as best indicators of GV during CGM was statistically significant:  $R = 0.42$  for SD ( $P = 0.005$ );  $R = 0.45$  for MAGE ( $P = 0.002$ ). The same applied for HbA1c ( $R = 0.72$ ,  $P < 0.001$ ), IG 2h after TM ( $R = 0.33$ ,  $P = 0.03$ ), IG peak during TM ( $R = 0.36$ ,  $P = 0.02$ ) and 2 h-AUC after TM ( $R = 0.41$ ,  $P = 0.006$ ). These correlations indicate a stable glycaemic pattern over time. Average IG concentration and 24h-AUC were not significantly correlated between baseline and FU. In contrast, there was a small but significant increase in mean HbA1c (baseline vs. FU:  $5.70$  vs.  $5.95\%$ ,  $P < 0.001$ ), SD ( $1.25$  vs.  $1.45$  mmol/l,  $P = 0.029$ ), IG 2h after TM ( $6.60$  vs.  $7.56$  mmol/l,  $P = 0.005$ ), IG peak during TM ( $8.55$  vs.  $9.88$  mmol/l,  $P = 0.001$ ) and average IG concentration ( $6.14$  vs.  $6.57$  mmol/l,  $P = 0.017$ ).

**Conclusion:** CGM under stable conditions reveals an individual pattern of glycaemic variability in well controlled T2D patients even over longer time periods. This might be of importance for an individualized treatment of type 2 diabetes. Despite a persistent good glycaemic control, most of CGM related glycaemic determinants slightly deteriorated during 4 years of follow-up.

*Supported by: Sanofi-Aventis Deutschland GmbH*

### 200

#### Standard deviation of glucose concentration in continuous glucose monitoring efficiently describes glycaemic variability in type 1 diabetic patients with complications

M. Prázný, J. Šoupal, M. Fajmon, J. Škrha jr., J. Škrha; Dept. of Internal Medicine 3, 1st Faculty of Medicine, Prague, Czech Republic.

**Background and aims:** Increased glycaemic variability (GV) may be associated with higher risk of microvascular complications (MVC) in Type 1 diabetes (T1DM). Continuous glucose monitoring systems are now widely available and can provide more data for the assessment of GV. Different parameters can be used for the description of GV and it is not clear which represents the best choice. The aim of the study was to compare GV in T1DM patients with and without MVC using different parameters.

**Materials and methods:** Thirty-two T1DM patients (mean age  $43 \pm 13$  yrs, duration of diabetes  $19 \pm 11$  yrs), of them 15 patients with and 17 without MVC (HbA1c  $71 \pm 9$  and  $66 \pm 13$  mmol/mol,  $p = 0.21$ , NS), participated in the study. Standard deviation (SD), coefficient of variation (CV), mean amplitude of glycaemic excursions with or without SD (MAGE and MAGE-SD) and continuous overlapping net glycaemic action with 1 and 4 hours time-intervals (CONGA-1 and -4) of glucose concentrations were calculated from CGMS records by a proprietary software. Vibration perception threshold (VPT) was measured by neurothesiometer (Bailey Instruments) and used as an estimate of diabetic neuropathy ( $0-15$  V normal,  $> 15$  V impaired VPT), retinopathy was examined by ophthalmoscopy and microalbuminuria (24hr urinary al-

bumin to creatinine ratio) was used as a marker of diabetic nephropathy. Data are mean $\pm$ SD.

**Results:** GV was higher in patients with retinopathy ( $n=13$ ) compared to patients without retinopathy (SD:  $4.1\pm0.7$  vs.  $3.4\pm0.9$ ,  $p=0.03$  and MAGE:  $7.0\pm1.1$  vs.  $5.9\pm1.4$ ,  $p=0.04$ ) although there was no significant difference in HbA1c between the groups. Similarly, GV was higher in patients with impaired VPT ( $n=12$ ) compared to the patients with normal VPT (SD:  $4.3\pm0.7$  vs.  $3.4\pm0.8$ ,  $p=0.002$ ; CV:  $0.44\pm0.06$  vs.  $0.38\pm0.07$ ,  $p=0.015$ ; MAGE:  $7.2\pm1.2$  vs.  $5.7\pm1.1$ ,  $p=0.001$ ; MAGE-SD:  $5.7\pm1.2$  vs.  $4.7\pm0.9$ ). Moreover, a positive association was observed between VPT and SD in all patients combined ( $r=0.53$ ,  $p=0.002$ ). Patients with microalbuminuria ( $n=7$ ) had significantly higher GV than patients without microalbuminuria (CV:  $0.46\pm0.07$  vs.  $0.39\pm0.07$ ,  $p=0.02$ ) even though HbA1c was nonsignificantly higher in the group without microalbuminuria ( $68\pm13$  vs.  $67\pm8$  mmol/mol, NS). In general, patients with any MVC had significantly higher GV compared to patients without complications (SD:  $4.1\pm0.7$  vs.  $3.4\pm0.9$ ,  $p=0.01$  and CV:  $0.44\pm0.06$  vs.  $0.37\pm0.07$ ,  $p=0.005$ ) although their glycemic control was comparable.

**Conclusion:** T1DM patients with any microvascular complication had significantly higher GV than the patients without complications although they did not differ in glycemic control. This finding supports the hypothesis that HbA1c may not describe the diabetes control completely and accurately. We conclude that SD represents an easy estimate of glycemic variability and in comparison with other more complex parameters it performs very well.

Supported by: Charles University in Prague

## 201

### Racial and ethnic differences in mean blood glucose, HbA<sub>1c</sub>, and estimated average glucose in patients with type 2 diabetes

B.H.R. Wolffenbuttel<sup>1</sup>, W.H. Herman<sup>2</sup>, H.H. Jiang<sup>3</sup>, M.A. Carey<sup>4</sup>, D.S. Hardin<sup>3</sup>

<sup>1</sup>University Medical Center, Groningen, Netherlands, <sup>2</sup>University of Michigan, Ann Arbor, USA, <sup>3</sup>Lilly USA, LLC, Indianapolis, USA, <sup>4</sup>PharmaNet/i3, Blue Bell, USA.

**Background and aims:** Recent studies have reported HbA1c differences across racial/ethnic groups. The A1C-Derived Average Glucose (ADAG) study recommended reporting HbA1c in estimated average glucose (eAG) equivalents. We aimed to assess the relationship between HbA1c and self-monitored mean blood glucose (SMBG) in different racial/ethnic groups, and to compare eAG with SMBG to determine whether the use of eAG is acceptable and feasible in different ethnic groups with type 2 diabetes.

**Materials and methods:** The DURABLE trial assessed the differences between 2 insulin starter regimens, and enrolled 2091 patients with type 2 diabetes, ages 30–80, from 11 countries. Full data are available for this analysis at baseline and after stabilisation of glycaemic control 6 months after initiation of insulin therapy, in the following racial/ethnic groups: Caucasian ( $n=1241$ , 63.9%), Asian ( $n=304$ , 15.7%), Hispanic ( $n=225$ , 11.6%), African Descent ( $n=109$ , 5.6%), and Other ( $n=62$ , 3.2%; not shown). A population regression equation was calculated for all racial subgroups using the mean of all SMBG values and mean HbA1c for each group. eAG was calculated from the ADAG linear regression equation. A mean blood glucose index (MBGI) was calculated as the difference between mean blood glucose (MBG) and eAG.

**Results:** Baseline mean  $\pm$  SD HbA1c was  $9.0 \pm 1.3\%$ , and MBG (via 7-point SMBG profile) was  $12.1 \pm 3.1$  mmol/l. There was a linear relationship between HbA1c and SMBG in all racial/ethnic groups, but for a given MBG, HbA1c was 0.3 and 0.4% higher in Hispanic patients and people of African Descent, respectively, compared to Caucasians. At MBG levels  $<11$  mmol/l, eAG overestimated the actual average blood glucose (BG), and at levels  $>11$  mmol/l, eAG underestimated the actual BG levels (Figure 1).

**Conclusion:** For a certain degree of glycaemia, actual HbA1c levels vary between the different racial/ethnic groups. eAG, calculated from HbA1c, is not a reliable surrogate for actually measured BG levels, and is therefore of limited clinical use.

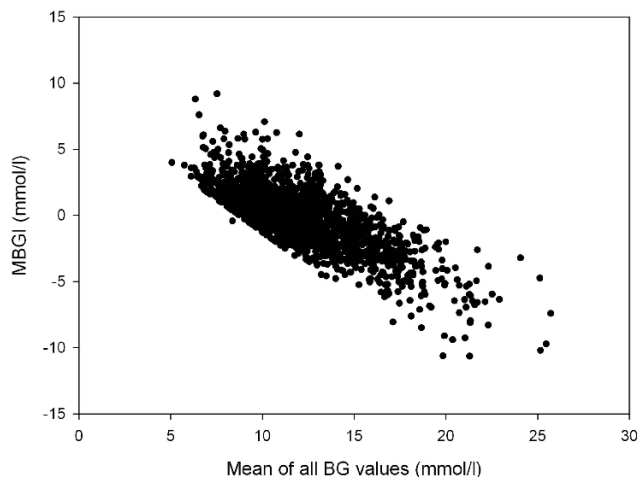


Figure 1 Relationship between mean blood glucose index (MBGI) and mean of all blood glucose (BG) values

Clinical Trial Registration Number: NCT00279201

Supported by: Lilly USA, LLC

## 202

### Monnier's hypothesis revisited: postprandial (PPG) vs fasting (FPG) hyperglycaemia at baseline and response to basal or premixed insulin stratified by HbA<sub>1c</sub> target achieved

A.J. Scheen<sup>1</sup>, J. Rosenstock<sup>2</sup>, H. Schmitt<sup>3</sup>, H.H. Jiang<sup>4</sup>, T. Ivanyi<sup>5</sup>

<sup>1</sup>CHU Liege, University of Liege, Belgium, <sup>2</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, USA, <sup>3</sup>Eli Lilly, Brussels, Belgium, <sup>4</sup>Eli Lilly, Indianapolis, USA, <sup>5</sup>Eli Lilly, Budapest, Hungary.

**Background and aims:** Monnier's cross-sectional study in Type 2 Diabetes (T2D), using 4-point glycaemic profiles during daytime, showed a greater contribution of PPG vs FPG to overall hyperglycaemia at lower HbA<sub>1c</sub>. We challenged this hypothesis, using a 24h glycaemic profile and comparing effect of a basal insulin, insulin glargine (G;  $n=1046$ ) with a premix insulin with a prandial component, insulin lispro mix 25 (25% insulin lispro, 75% insulin lispro protamine suspension, LM25;  $n=1045$ ).

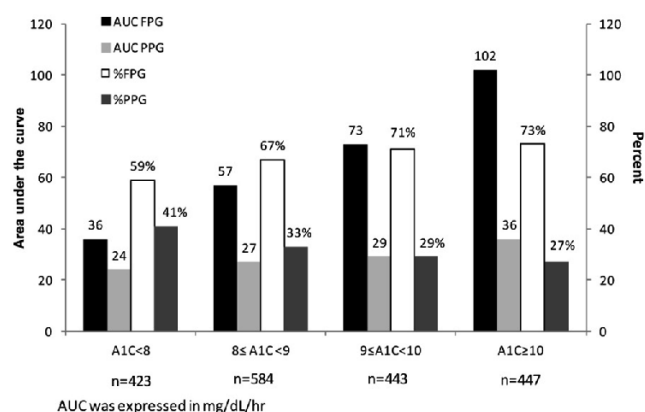
**Materials and methods:** In the initiation phase of DURABLE study [T2D on  $\geq 2$  OADs], patients were randomized to once-daily G or twice daily LM25 for 24 weeks. Relative 24h contributions of PPG and FPG to overall hyperglycaemia were calculated from 7-point glucose profiles with Area Under the Curve (AUC) for FPG between 100 mg/dL and fasting glycaemia and for PPG above the line projected from fasting glycaemia.

**Results:** At baseline, with increasing HbA<sub>1c</sub>, contributions of FPG to total AUC increased from 59 to 73% and PPG decreased from 41 to 27% (Figure 1). FPG AUC increased linearly with HbA<sub>1c</sub> while PPG AUC increased only slightly. At endpoint, both LM25 and G lowered FPG AUC but only LM25 lowered PPG AUC. Below 8% and between 8–9% HbA<sub>1c</sub> at baseline, LM25 allowed a significantly higher % of patients to reach target  $<7\%$  HbA<sub>1c</sub> vs G (72 vs 62% and 52 vs 43%, respectively). Similar % of patients reached target HbA<sub>1c</sub> with LM25 and G in quartiles HbA<sub>1c</sub> 9–10% (31 vs 28%) and  $\geq 10\%$  (28 vs 25%). Comparison of patients reaching target, or not, per quartile, showed baseline HbA<sub>1c</sub>, FPG and FPG AUC were similar but decreases were greater in those reaching target, allowing lower endpoint values. The higher the HbA<sub>1c</sub> baseline quartile, the greater was the absolute decrease in HbA<sub>1c</sub> for both insulins. Patients with baseline HbA<sub>1c</sub>  $<8\%$ , not reaching target with both insulins, had a higher % of PPG AUC at baseline ( $\sim 49$  vs  $\sim 37\%$ ), suggesting they could benefit from an insulin regimen with a higher prandial percentage or higher insulin doses. Patients not reaching target had a slightly higher insulin dose but lower rate of hypoglycaemia, suggesting insulin resistance, and possibly, an unwillingness to increase dose further and/or poor compliance to lifestyle and the insulin regimen.

**Conclusion:** At baseline, the relative contribution of PPG vs FPG decreased with higher HbA<sub>1c</sub>, confirming Monnier's findings, and with a relevant role of PPG only at lower HbA<sub>1c</sub> and hence a higher % of patients reaching target with LM25 at low HbA<sub>1c</sub> quartiles. However, FPG AUC predominated at all HbA<sub>1c</sub> quartiles and was potentially amenable to further improvements. In all HbA<sub>1c</sub> quartiles, there was a lower decrease in FPG and FPG AUC in patients

above target, suggesting that insulin dose could be increased further or then patients need to be advanced to more intensive regimens.

**Figure 1: AUC FPG vs. AUC PPG at Baseline for all Randomized Patients (N=1897)**



Clinical Trial Registration Number: NCT00279201

Supported by: Eli Lilly and Company

## 203

### The extent of and variation in mortality among inpatients with diabetes

N. Holman;

Diabetes Health Intelligence, Yorkshire and Humber Public Health Observatory, York, UK.

**Background and aims:** It is known that people with diabetes have higher hospital admission and mortality rates than their peers. However, little is known about the relative risk of inpatient mortality among patients whether local variation is due to different patient populations and/or in healthcare services. This work aimed to assess the additional risk of dying amongst inpatients with diabetes and hospital variation in this risk across England.

**Materials and methods:** The record of all hospital admissions (Hospital Episode Statistics) was used to identify all hospital admissions between April 2009 and March 2011. Admissions relating to pregnancy, childbirth, babies born in hospital and those in specialist hospitals were excluded. Binary regression models were used to assess the impact of age, sex, social deprivation, method of admission, reason for admission and type of hospital on the odds of dying nationally and for individual hospital trusts. Two hospital level standardised mortality ratios were calculated - one compared in the hospital trust to all inpatients with diabetes in England and one adjusted for the pattern of mortality among inpatients without diabetes in the same hospital trust. The former standardised mortality ratio was correlated with a national measure of all cause hospital mortality (Summary Hospital-level Mortality Indicator or SHMI).

**Results:** A total of 13,150,750 hospital admissions in 148 hospital trusts meet the inclusion criteria. Of these 1,474,363 (11.2%) were in patients with diabetes. The crude mortality rate was 4.9% for inpatients with diabetes compared to 3.0% for those without diabetes (additional risk 76.2%). Adjusting for age and sex reduced the additional risk of death to 14.4% ( $p < 0.005$ ). Adding method of admission, reason for admission and the type of hospital further reduced the additional risk to 9.9% ( $p < 0.005$ ). When patients were split into quintiles based on the social deprivation score of their home postcode, there was a clear social deprivation gradient in the adjusted risk of death for inpatients without diabetes after adjustment for age, sex, method of admission, reason for admission and type of hospital trust (RR compared to least deprived quintile - 1.099, 1.084, 1.056 and 1.045, all  $p < 0.005$ ). However, there was no clear gradient amongst inpatients with diabetes (RR compared to least deprived quintile - 0.964  $p = 0.384$ , 1.005  $p = 0.108$ , 1.024  $p < 0.005$ , 1.025  $p < 0.005$ ). When mortality among inpatients with diabetes was compared to England 23 hospital trusts (15.5%) were above the 95% CI and 8 (5.4%) were above the 99.9% CI. There was a significant positive correlation between the mortality ratio standardised to all inpatients with diabetes in England and SHMI ( $r = 0.674$ ,  $r^2 = 0.451$ ,  $p < 0.005$ ). When mortality among inpatients with diabetes is standardised using patients without diabetes from the same hospital variation persists with five (3.4%) hospital trusts having ratios above the 95% CI and 13 (8.8%) having ratios lower than the 95% CI.

**Conclusion:** This analysis found that inpatients with diabetes had a 9.9% higher risk of mortality (after case mix adjustment) than inpatients without diabetes. Unlike those without diabetes, there was no social deprivation gradient in inpatient mortality among inpatients with diabetes. Some of the variation (45%) in inpatient mortality among those with diabetes can be explained by the pattern of all cause mortality but when local adjustments are made some hospital trusts show significantly different mortality ratios.

Supported by: NHS Diabetes

## 204

### State of diabetes-related trials in the clinicaltrials.gov dataset

J.B. Green<sup>1,2</sup>, W. Lakey<sup>1,2</sup>, K. Barnard<sup>1,2</sup>, B. Batch<sup>1,2</sup>, M. Bethel<sup>3,4</sup>, K. Chiswell<sup>5</sup>, A. Tasneem<sup>5</sup>;

<sup>1</sup>Division of Endocrinology, Metabolism, and Nutrition, Duke University Medical Center, Durham, USA, <sup>2</sup>Durham Veterans Affairs Medical Center, USA, <sup>3</sup>Diabetes Trials Unit, University of Oxford, UK, <sup>4</sup>Duke University Medical Center, Durham, USA, <sup>5</sup>Duke Clinical Research Institute, Durham, USA.

**Background and aims:** Trials which assess interventions to prevent diabetes and its complications and enhance our ability to care for all affected are needed. It is unclear if the scope of current clinical trials will adequately address deficiencies in our understanding of diabetes care.

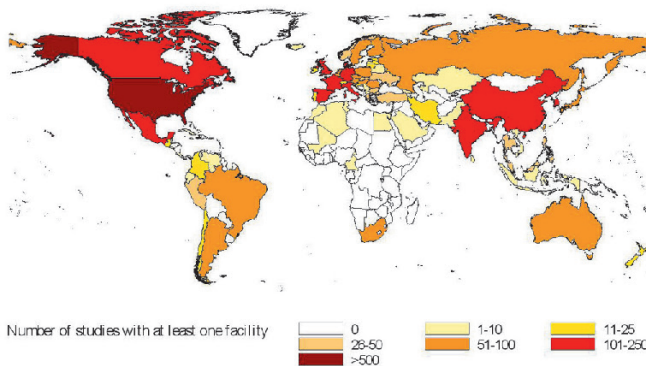
**Materials and methods:** ClinicalTrials.gov is a registry which contains information entered by sponsors and investigators conducting clinical trials in the US and around the world. A dataset of 96,346 studies registered in ClinicalTrials.gov was downloaded on September 27, 2010. The diabetes study subset of 2,484 interventional trials was created by selecting those trials with relevant disease condition terms. The set of terms chosen was intentionally broad in order to capture trials enrolling patients with diagnosed diabetes as well as related conditions such as pre-diabetes or insulin resistance.

**Results:** Diabetes-related trials comprised 4.8% of the 40,970 interventional trials overall. 74.8% of the diabetes trials had a primarily therapeutic purpose while 10% had a preventative purpose. Listed interventions included drugs (63.1%) and behavioral (11.7%). Most trials were to enroll  $\leq 500$  (91%) or  $\leq 100$  (58.6%) subjects, with mean/median times to completion of 1.8/1.4 years. Small percentages of trials targeted individuals aged 18 or younger (3.7%) or 65 or older (0.6%), while 30.8% excluded patients older than 65 and the majority excluded those over 75. Funding included industry (50.9%), NIH (7.5%), or other with most being single-center trials of other sponsorship (37.7%) or industry-funded multi-center studies (27.4%). Of the 2500 primary outcomes listed in free text, 51 (0.02%) included mortality or significant cardiovascular complications such as myocardial infarction or stroke. Regions where studies had facilities included North America (56.1%), Europe (33.5%), Eastern Asia (13.5%), and South America (7%). 40.5% of trials had facilities in only the US, 49.7% were outside the US, and 9.8% were conducted in the US and other regions. Distribution of diabetes trials by country is outlined in the attached figure.

**Conclusion:** The majority of diabetes-related trials include relatively small numbers of patients, exclude those at extremes of age, are of short duration, involve drug therapy rather than studying prevention or non-drug interventions, and do not focus upon significant cardiovascular outcomes. The distribution of trials does not correlate with the prevalence of diabetes in many countries, perhaps most notably in the Middle East, Latin America, Southern Africa, and the Russian Federation. Recently registered diabetes trials may not sufficiently address important diabetes care issues or involve affected populations.



## Distribution of diabetes studies by country



Supported by: US FDA grant U19FD003800 awarded to Duke University for the CTTI

## OP 35 Regulators of adipose tissue expansion

### 205

**Adipocyte-specific deletion of Janus kinase 2 (JAK2) leads to increased adiposity, reduced energy expenditure and age-dependent glucose intolerance**

S.Y. Shi<sup>1,2</sup>, C.T. Luk<sup>1,2</sup>, S.A. Schroer<sup>2</sup>, K.-U. Wagner<sup>3</sup>, M. Woo<sup>2,4</sup>;

<sup>1</sup>Institute of Medical Science, University of Toronto, Canada, <sup>2</sup>Toronto General Research Institute, University of Toronto, Canada, <sup>3</sup>University of Nebraska Medical Center, Omaha, USA, <sup>4</sup>Department of Medicine, St. Michael's Hospital, Toronto, Canada.

**Background and aims:** Adipocytes were once thought to be inert energy storage depots, but are now recognized as active endocrine cells that secrete hormones and cytokines to regulate energy balance and metabolic homeostasis. Alterations in adipocyte development and/or function have been implicated in the pathophysiology of metabolic diseases, particularly type 2 diabetes. A better understanding of adipocyte biology is therefore crucial given the growing worldwide epidemic of obesity and diabetes. The Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway mediates signal transduction of numerous cytokines and hormones that regulate adipocyte development and function. Several adipokines secreted by adipocytes also utilize this signaling pathway, illustrating the physiological importance of JAK-STATs in adipocyte biology. This study aims to investigate the metabolic roles of JAK2 in adipocytes.

**Materials and methods:** We utilized an *in vivo* genetic approach and generated mice with deletion of JAK2 specifically in fat cells (F-JAK2 KO) by breeding mice with their *Jak2* gene flanked by loxP sites with mice expressing the *Cre* recombinase driven by the aP2 promoter.

**Results:** F-JAK2 KO mice gradually gained more body weight on a chow diet starting at 2 months of age, and by 5 months, they weighed about 60% more than control littermates ( $p < 0.001$ ). Relative weight of isolated subcutaneous, visceral and brown fat pads from 24-30-week-old F-JAK2 KO mice was 2 to 6 fold higher than controls, suggesting that the higher body weight in F-JAK2 KO mice was due to increased adiposity. Next, we placed mice individually in metabolic chambers to measure their energy balance status. We found no difference in absolute daily food intake ( $2.25 \pm 0.36$  in F-JAK2 KO vs  $2.07 \pm 0.23$  g/day in controls;  $p = 0.72$ ). F-JAK2 KO mice were also comparable to controls in terms of the fuel source utilized, as evidenced by a similar respiratory exchange ratio. On the other hand, rates of  $O_2$  consumption and  $CO_2$  production were about 30% lower in F-JAK2 KO mice ( $p < 0.001$ ), consistent with reduced energy expenditure. This may be explained, at least in part, by reduced physical activity ( $p < 0.05$ ). We next determined the metabolic consequences of adipocyte JAK2 deletion. At 2 months of age, despite being significantly obese, F-JAK2 KO mice did not display abnormalities in fasting blood glucose ( $3.9 \pm 0.5$  in F-JAK2 KO vs  $4.0 \pm 0.4$  mM in controls;  $p = 0.86$ ), glucose tolerance or insulin sensitivity. Even at 4-6 months of age, F-JAK2 KO mice maintained normal levels of random and fasting blood glucose. However, when given an i.p. glucose challenge, they appeared to be more intolerant than controls (AUC  $1392.0 \pm 193.1$  in F-JAK2 KO vs  $913.2 \pm 40.7$  in controls;  $p < 0.05$ ) and there was also a trend towards reduced insulin sensitivity as demonstrated by i.p. insulin tolerance test.

**Conclusion:** Taken together, our results implicate a critical role of JAK2 in regulation of adipocyte biology and whole-body glucose metabolism. Targeting the JAK-STAT pathway may therefore provide a novel therapeutic strategy for the treatment of type 2 diabetes.

Supported by: CIHR MOP-191501, CDA Grant-in-Aid and BBDC

### 206

**Essential role of adipocyte focal adhesion kinase in mediating insulin resistance**

C.T. Luk<sup>1,2</sup>, S.Y. Shi<sup>1,2</sup>, E.P. Cai<sup>1,2</sup>, T.S. Sivasubramaniam<sup>2</sup>, S.A. Schroer<sup>2</sup>, M. Woo<sup>2,3</sup>;

<sup>1</sup>Institute of Medical Science, <sup>2</sup>Toronto General Research Institute,

<sup>3</sup>Department of Medicine, Toronto, Canada.

**Background and aims:** Adipose tissue extracellular matrix (ECM) has recently been shown to be important in whole body glucose homeostasis but little is known about the mechanism by which signals from the ECM are

translated into adipocytes. Focal adhesion kinase (FAK) is a ubiquitously expressed tyrosine kinase involved in integrin signalling and essential for development and proliferation. Interestingly, inhibition of FAK has been implicated in insulin resistance by studies showing that disruption of FAK in hepatocytes and myocytes impairs glycogen production and actin-mediated GLUT4 translocation. Although FAK has been shown to be phosphorylated in response to insulin in adipocyte cell lines, its role in adipocyte glucose homeostasis and energy metabolism has not been established. The aim of this study was to assess the role of adipocyte FAK in insulin resistance.

**Materials and methods:** To generate a novel adipose-specific FAK knock-out mouse (*aP2Cre<sup>+</sup>FAK<sup>fl/fl</sup>*), transgenic mice with adipocyte protein 2 (*aP2*) promoter-driven CRE recombinase expression in adipocytes were crossed with *FAK<sup>fl/fl</sup>* mice. To further investigate the role of FAK in the setting of diabetes and obesity, at 8 weeks of age, *aP2Cre<sup>+</sup>FAK<sup>fl/fl</sup>* mice were placed on high-fat diet for 16 weeks.

**Results:** By 12 weeks of age, *aP2Cre<sup>+</sup>FAK<sup>fl/fl</sup>* mice on chow diet had higher fasting blood glucose levels than wild-type littermate controls ( $7.4 \pm 0.4$  vs  $5.6 \pm 0.7$  mmol/L,  $n=9$ ,  $p<0.05$ ) despite no difference in total body weight. While glucose tolerance was preserved on intraperitoneal glucose tolerance testing, *aP2Cre<sup>+</sup>FAK<sup>fl/fl</sup>* were more insulin resistant on insulin tolerance testing ( $p<0.05$ ), and had higher fasting insulin levels ( $0.73 \pm 0.11$  vs  $0.35 \pm 0.06$  ng/mL,  $n=4$ ,  $p<0.05$ ). Similarly, leptin gene expression and serum leptin levels were increased (5.4-fold,  $n=7$ ,  $p<0.05$ ;  $1.58 \pm 0.12$  vs  $0.83 \pm 0.06$  ng/mL,  $n=6$ ,  $p<0.05$ ). Analysis of body composition by necropsy showed increased white adipose tissue fat pad weight (+71% for perigonadal fat pad,  $n=4$ ,  $p<0.05$ ). Differences in body composition could not be accounted for by differences in energy homeostasis, as measured by food intake, ambulatory activity, respiratory exchange ratio or oxygen consumption. However, sterol regulatory element binding protein-1c (SREBP-1c) and macrophage-specific F4/80 gene expression were increased (2.9-fold,  $n=7$ ,  $p<0.05$ ; 2.6-fold,  $n=7$ ,  $p<0.05$ ). Surprisingly, on high-fat diet, *aP2Cre<sup>+</sup>FAK<sup>fl/fl</sup>* mice continued to be insulin resistant but had 29% lower body weight ( $n=5$ ,  $p<0.05$ ), suggesting impairment of FAK may contribute to impaired adipocyte growth, reminiscent of lipodystrophy.

**Conclusion:** Overall, knockdown of adipocyte FAK resulted in insulin resistance and increased adiposity under basal conditions but decreased weight gain on high-fat diet. These results demonstrate a new and important role for FAK in adipocyte mediation of adiposity and whole body insulin sensitivity. *Supported by: CIHR MOP-191501, CDA Grant-in-Aid and BBDC*

## 207

### Mice with beta cell specific overexpression of CART exhibit altered adipocyte lipid metabolism

E. Banke<sup>1</sup>, M. Riva<sup>2</sup>, M. Ländin<sup>2</sup>, A.-H. Thorén Fischer<sup>2</sup>, E. Degerman<sup>1</sup>, N. Wierup<sup>2</sup>

<sup>1</sup>Experimental Medical Sciences, Lund, <sup>2</sup>Clinical Sciences in Malmö, Sweden.

**Background and aims:** Cocaine and amphetamine regulated transcript (CART) is a regulatory peptide expressed in the central and peripheral nervous systems, as well as in the pancreatic islets. CART deficient mice exhibit islet dysfunction, impaired insulin secretion and increased body weight. On the other hand, islet CART is upregulated in type 2 diabetes patients and in animal models of type 2 diabetes. Our recent studies showed that CART is expressed also in human and rodent adipocytes and that CART affects several processes important for adipocyte function. Depending on the hormonal context, the effects of CART are insulin-like or insulin antagonistic. The aim of this study was to investigate the effect of increased levels of CART on adipocyte lipid metabolism.

**Materials and methods:** To study the consequence of an increased expression of CART in beta cells on whole body metabolism, transgenic mice with beta cell-specific (under the control of the PDX-1 promoter) overexpression of CART (CARTtg) was constructed. Characterisation of the CARTtg mice is ongoing. Here we focus on the effects of increased levels of CART on adipocyte lipid metabolism. Primary adipocytes from wild type and CARTtg mice, kept on either control or high fat diet, were isolated and lipolysis was measured after stimulation with the beta adrenergic receptor agonist isoprenaline with or without insulin.

**Results:** CARTtg mice both in the control and high fat diet group displayed significantly lower basal lipolysis compared to wild type mice. Also, CARTtg mice were more responsive to isoprenaline-induced lipolysis in both diet groups. Furthermore, the ability of insulin to inhibit lipolysis was improved in CARTtg mice on control diet compared to wild type mice. These data are in agreement with recent in vitro studies in rat adipocytes using exogenous CART.

**Conclusion:** Our data suggest a role for CART in cross talk between beta cells and adipocytes. Elevated levels of CART in beta cells alters adipocyte lipid metabolism by making them more responsive to adrenergic stimuli as well as enhancing adipocyte insulin sensitivity. Ongoing studies are investigating whether CART levels are also increased systemically.

*Supported by: FLÄK, VR, Novo Nordisk Foundation, Crafoord Foundation, Pahlsson Foundation*

## 208

### RB1 mRNA, protein and activity in association with adipogenic capacity of human adipose tissue

J.M. Moreno-Navarrete<sup>1</sup>, P. Petrov<sup>2</sup>, M. Serrano<sup>1</sup>, F. Ortega<sup>1</sup>, J. Ribot<sup>2</sup>, W. Ricart<sup>1</sup>, A. Palou<sup>2</sup>, M. Bonet<sup>2</sup>, J. Fernández-Real<sup>1</sup>

<sup>1</sup>Hospital of Girona, <sup>2</sup>Universitat de les Illes Balears, Palma de Mallorca, Spain.

**Background and aims:** Retinoblastoma (Rb1), the extensively known tumor suppressor gene, has been described as an essential player in mouse white adipocyte differentiation, being a negative modulator of brown adipocyte differentiation. To our knowledge, no studies have explored Rb1 in human adipose tissue or adipocytes. We aimed to investigate retinoblastoma protein family [RB1, RBL2 (p130) and RBL1 (p107)] in human adipose tissue and adipocytes according to obesity and insulin sensitivity.

**Materials and methods:** RB1, RBL2 and RBL1 gene expressions (Real Time PCR) were analysed in two independent cohorts, in 154 adipose tissue samples (77 visceral and 77 subcutaneous depots) from participants with different degrees of obesity and in a second cohort of 64 adipose tissues (32 visceral and 32 subcutaneous depots) from morbidly obese participants with different degrees of insulin action (measured with euglycemic clamp). The expression of these genes also was studied during human and 3T3-L1 (mouse cell line) adipocyte differentiation in a time course experiment. Rb activity was calculated measuring  $\text{phospho}^{\text{Thr821}}\text{Rb}$  and  $\text{total Rb}$  in adipose tissue samples and during adipogenesis. Finally, the effects of Rb knockdown in differentiated 3T3-L1 adipocytes were tested using two different methods (permanent silencing with lentiviral particles and transient silencing with electroporation).

**Results:** RB1 and RBL2 gene expressions were negatively associated with BMI in both visceral (VAT) and subcutaneous (SAT) adipose tissues and positively associated with PPAR $\gamma$  and IRS1. In SAT, these genes were also negatively associated with leptin and positively with FASN and ACC gene expression. RBL1 gene expression was not associated with metabolic parameters. Similar to RB1 gene expression, RB1 protein levels and activity in adipose tissue decreased in association with obesity. Interestingly, RB1 was negatively associated with fasting insulin and HOMA $_{\text{IR}}$ . RB1 and RBL2 increased significantly during adipocyte differentiation in parallel to adipogenic genes. RBL1 tended to decrease during adipocyte differentiation. RB1 permanent knockdown (60%) decreased adipogenic gene expression (PPAR $\gamma$ , adiponectin, FASN, ACC, CEBP $\alpha$ , FABP4, IRS1 and GLUT4) whereas increased brown-like adipocyte related genes (PRDM16 and UCP1) during adipocyte differentiation. RB1 transient knockdown (65%, 32 h), in fully differentiated adipocytes led to loss of the adipogenic phenotype, decreasing significantly PPAR $\gamma$ , adiponectin, FASN and FABP4 gene expression, while no effects were observed in brown-like adipocyte related genes.

**Conclusion:** To the best of our knowledge, this is the first study showing a strong relationship among RB1, RBL2 and adipogenesis in human adipose tissue and adipocytes, being RB1 mRNA, protein level and activity decreased in association with obesity. RB1 permanent knockdown confirmed findings in knockout mice which showed the role of RB1 on white adipocyte differentiation. In addition, RB1 was necessary to maintain adipogenic characteristics in fully differentiated adipocytes.

*Supported by: ISCIII and CIBERobn*

## 209

**Visceral fat accumulation during overfeeding is related to subcutaneous adipose tissue characteristics in humans**

M. Laville<sup>1,2</sup>, M. Alligier<sup>1,3</sup>, L. Gabert<sup>1,4</sup>, E. Meugnier<sup>4,5</sup>,  
S. Lambert-Porcheron<sup>5,6</sup>, B. Morio<sup>6,7</sup>, A. Vidal Puig<sup>7</sup>, H. Vidal<sup>8</sup>;

<sup>1</sup>Lyon 1 University, Pierre-Bénite, France, <sup>2</sup>University of Cambridge, UK, <sup>3</sup>CRNH Auvergne, Clermont-Ferrand, France, <sup>4</sup>Inserm U MR 1060 CARMEN, Oullins, France, <sup>5</sup>CRNH RA, Pierre-Bénite, France, <sup>6</sup>INRA UMR1019, Clermont-Ferrand, France, <sup>7</sup>Institute of Metabolic Science, Cambridge, UK, <sup>8</sup>Inserm U MR 1060 CARMEN, Pierre-Bénite, France.

**Background and aims:** The hypothesis of a limited expansion of subcutaneous adipose tissue during weight gain provides an attractive explanation for the reorientation of excess lipids towards ectopic sites, contributing to metabolic complications. We investigated how the characteristics of subcutaneous adipocytes influence the partition of lipids towards abdominal fat depots during an experimental overfeeding

**Materials and methods:** A 56 day overfeeding (+760 Kcal/day) experiment was realised in 41 healthy non-obese volunteers. Abdominal adipose tissue depot volume was determined by MRI and postprandial fatty acids spill-over was measured during test meal labelled with [d31]-deuterated palmitic acid. Gene expression was realised in sub-cutaneous adipose tissue biopsies by RT-PCR

**Results:** Overfeeding led to a  $2.5 \text{ Kg} \pm 0.2$  ( $p = 0.001$ ) weight gain. Both abdominal subcutaneous ( $+14 \pm 2\%$ ,  $p = 0.001$ ) and visceral ( $+22 \pm 5\%$ ,  $p = 0.001$ ) increased significantly during overfeeding but with large interindividual variations in the volume. There was no relationship between the relative expansion of these two fat depots, but the increased in visceral fat was positively associated with the magnitude of the postprandial fatty acid spill-over (AUCTION-T300 :  $r = 0.66$ ,  $p = 0.02$ ). The regulation of lipid storage-related genes (SREBP1C, DGAT2 and CIDEA) was defective in the subcutaneous fat of the subjects exhibiting the largest accumulation in visceral depot. Conversely, high subcutaneous fat gainers had increased induction of lipogenic genes and down-regulation of the lipolytic genes HSL and ATGL in their subcutaneous fat.

**Conclusion:** We demonstrate that the partition of lipids towards the abdominal fat depots during overfeeding in non-obese subjects is associated with molecular and functional characteristics of the subcutaneous adipose tissue. These results lend support to the adipose tissue expandability theory and provide evidence that defects in adipose tissue expandability can be detected early on during the evolution towards obesity with proper functional challenges. Further studies are now required to validate that controlled metabolic challenges such as overfeeding and lipid tolerance tests could provide new insights for the identification of subjects at risk of obesity complications.

Clinical Trial Registration Number: NCT00905892

Supported by: ANR and PHRC

## 210

**RBP4 and its membrane receptor stra6 control adipogenesis by regulating cellular retinoid homeostasis**

M. Schupp<sup>1</sup>, J. Raila<sup>2</sup>, N. Witte<sup>1</sup>, N. Tuvia<sup>1</sup>, M. Muenzner<sup>1</sup>;

<sup>1</sup>Department of Endocrinology, Diabetes, and Nutrition, Charité-University Medicine Berlin, <sup>2</sup>Department of Physiology and Pathophysiology, University of Potsdam, Germany.

**Background and aims:** The understanding of adipocyte differentiation has important implications for the treatment of obesity. Retinoids inhibit adipogenesis by activating the retinoic acid receptor (RAR)  $\alpha$ . However, the physiologic regulation of retinoid levels in precursor cells is largely unknown. We investigated the function of retinol-binding protein (RBP) 4 and its recently identified membrane receptor stra6 during adipocyte differentiation.

**Materials and methods:** We applied cellular gain-and loss-of-function studies to adipocyte precursor cells by electroporation of siRNA and retroviral overexpression of stra6. Furthermore, cells were differentiated in the presence of retinol-bound (holo) or unbound (apo) recombinant RBP4 protein produced in *E. coli*. Cellular retinoid levels were determined by HPLC. RAR  $\alpha$  activity was assessed by the electroporation of luciferase-coupled reporter constructs. The expression of RAR  $\alpha$  target-genes was measured by qPCR.

**Results:** We found that the RBP4 membrane receptor stra6 protein is expressed in preadipocytes and increases during differentiation. Depletion of stra6 in precursor cells inhibited, whereas stra6 over-expression enhanced

differentiation. These effects were dependent on retinol/ RBP4, suggesting that stra6 regulates RAR  $\alpha$  ligand availability. Accordingly, RBP4 reduced cellular retinoid levels, RAR  $\alpha$  activity and its target-gene expression, and enhanced differentiation in a stra6 dependent manner only when present in its retinol-free apo-form. Holo-RBP4, in contrast, induced RAR  $\alpha$  target-genes and blocked differentiation.

**Conclusion:** Our data demonstrate opposing effects of apo- and holo-RBP4 on differentiation and show that RAR  $\alpha$ -activating retinoids in precursor cells need to efflux via a stra6/apo-RBP4 dependent mechanism for adipogenesis to occur. These findings reveal an important metabolic aspect of the retinoid homeostasis that may help us to develop novel retinoid- and RBP4-based therapies to treat obesity and type 2 diabetes.

Supported by: DFG Emmy-Noether SCHU 2546/1-1 and EU Career Integration Grant CIG 291867



## OP 36 Non-coding RNAs in the beta cells

211

### Role of the microRNA miR-338-3p in compensatory beta-cell mass expansion during pregnancy and obesity

C. Jacovetti<sup>1</sup>, A. Abderrahmani<sup>2</sup>, R. Regazzi<sup>1</sup>;<sup>1</sup>Department of Cell Biology and Morphology, Lausanne, Switzerland,<sup>2</sup>Institut Européen de Génomique de Lille, France.

**Background and aims:** Pregnancy and obesity are associated with insulin resistance and increased insulin needs. To preserve glucose homeostasis, the rise in insulin demand is compensated by expansion of the pancreatic  $\beta$ -cell mass. The molecular mechanisms underlying this phenomenon are still poorly understood. microRNAs constitute a large class of non-coding RNAs that play major roles in the control of gene expression by partially pairing to the 3'UTR of target mRNAs thereby inhibiting their translation and/or stability. The aim of this project is to determine the role played by microRNAs in compensatory  $\beta$ -cell expansion in a context of insulin resistance.

**Materials and methods:** We used expression profiling methods to search for changes in the level of microRNAs in the islets of insulin resistant animal models. Differential microRNA expression was verified by quantitative real-time PCR. The functional impact of selected microRNAs on insulin secretion, cell proliferation and survival was studied by modifying their expression in the insulin-secreting cell lines MIN6 and INS832/13 and in dissociated rat and human islet cells. Real-time PCR was used to determine the mechanisms regulating the levels of the microRNAs and of the genes controlled by the microRNAs.

**Results:** We identified a microRNA, miR-338-3p, which is down-regulated in the islets of different animal models displaying compensatory  $\beta$ -cell mass expansion such as pregnant rats, high fat fed mice and 6-week-old obese *db/db* mice. We found that reduction of miR-338-3p expression in rat and human islets could be achieved by incubation with estradiol or with the GLP-1 analogue exendin-4. Specific inhibition of miR-338-3p activity increased the fraction of proliferating  $\beta$ -cells, and promoted  $\beta$ -cell survival in the presence of apoptotic stimuli without affecting the capacity of the cells to release insulin in response to glucose. Moreover, knock-down of miR-338-3p in rat and human islet cells resulted in a decrease in the expression of apoptotic genes and up-regulation of anti-apoptotic and pro-proliferative genes.

**Conclusion:** We showed that blockade of miR-338-3p, a microRNA which is decreased in islets of animals displaying insulin resistance, permits to increase proliferation and survival of  $\beta$ -cells, suggesting its potential contribution to  $\beta$ -cell mass expansion associated to pregnancy and obesity. Our data could pave the way for the development of new microRNA-based strategies aiming at promoting the  $\beta$ -cell regenerative capacities and at preserving an appropriate functional  $\beta$ -cell mass.

Supported by: FNS, EFSD/MSD grant, SFD

212

### An islet-specific long non-coding RNA (lncRNA) expressed from the Pdx1 locus regulates Pdx1 activity

T.J. Pullen, G.A. Rutter;

Cell Biology / Div. Diabetes, Endocrinology &amp; Metabolism, Imperial College London, UK.

**Background and aims:** Maintained expression of Pancreatic and duodenal homeobox 1 (*Pdx1*) is required for normal beta cell function, as reflected by the defects in insulin secretion caused by *Pdx1* haploinsufficiency in MODY4 patients and mouse models. Although much work has elucidated a network of transcription factors regulating *Pdx1* expression, there is evidence that epigenetic factors around the *Pdx1* promoter can downregulate its expression, consequently interfering with beta cell function. There is also increasing evidence that long non-coding RNAs (lncRNAs) can interact with chromatin modifying complexes to target epigenetic marks to particular genomic loci. We therefore aimed to identify lncRNAs expressed in islets from the loci of transcription factors important for islet development and function, and determine whether they can regulate the expression of the corresponding protein-coding gene.

**Materials and methods:** Predicted non-coding transcripts arising <5 kb from key islet transcription factor loci were identified in the mouse genome

(Ensembl release 57). Expression levels of these lncRNAs and corresponding transcription factor mRNAs were determined using high-throughput RNA sequencing (RNA-seq) data of islets prepared from five C57Bl/6 mice. The lncRNA from the *Pdx1* locus (*Pdx1-asRNA*) was cloned by RT-PCR from mouse islet RNA, and three shRNAs were designed to target it. The effect of expressing cDNA of, or shRNA against *Pdx1-asRNA* on endogenous *Pdx1* activity was assayed in the MIN6 beta cell line by luciferase reporter assay using a 6xSMS-TAAT1 element.

**Results:** Of eight transcription factor/lncRNA pairs for which expression data were available, four showed clear expression of both mRNA and lncRNA in mouse islets (*Pdx1*, *Nkx2.2*, *Pax6*, *Hnf1a*). Consistent with previous reports of the relatively low abundance of lncRNAs, the expression levels of these lncRNAs was substantially lower than the corresponding mRNA at  $13 \pm 2\%$  (mean  $\pm$  SEM,  $n=4$ ). Of the remaining loci, one (*Foxa2*) showed substantial expression of the mRNA (>30 RPKM [Reads Per Kilobase of exon model per Million mapped reads]) but not lncRNA (<2 RPKM). The other three transcription factors (*Hnf1b*, *Ptf1a*, *Onecut1*) are important during pancreatic development but not expressed in mature islets and showed no substantial expression of either mRNA or lncRNA. The lncRNA expressed from the opposite strand of the *Pdx1* locus starts in the single intron of *Pdx1* although there is no sequence overlap between the mature lncRNA and mRNA ruling out a direct interaction between the two transcripts. qRT-PCR in eight mouse tissues showed that expression of both *Pdx1* and *Pdx1-asRNA* were restricted to islets. Whereas overexpression of *Pdx1-asRNA* exerted no significant effect on *Pdx1* activity as assessed by luciferase assay in MIN6 cells (overexpression vs empty vector control:  $0.111 \pm 0.002$  vs  $0.112 \pm 0.003$  RLU, mean  $\pm$  SEM  $n=3$   $p=0.728$ ), shRNAs against *Pdx1-asRNA* significantly decreased *Pdx1* activity ( $\alpha$ -*Pdx1-asRNA* shRNA vs  $\alpha$ -GFP shRNA control:  $0.066 \pm 0.002$  vs  $0.114 \pm 0.007$ , mean  $\pm$  SEM  $n=3$ ,  $p=0.002$ ).

**Conclusion:** We show here that lncRNAs are co-expressed from the loci of several key beta cell transcription factors. We demonstrate that the lncRNA expressed from the *Pdx1* locus in an islet-specific manner is required for the maintenance of normal *Pdx1* activity, and thus potentially for preserving beta cell mass and function.

Supported by: Wellcome Trust

213

### The regulatory role of miR-375 in the pancreatic beta cell secretome

S. Tattikota, M. Sury, T. Rathjen, C. Becker, M. Selbach, M. Poy;

Max Delbrück Centre for Molecular Medicine, Berlin, Germany.

**Background and aims:** The MicroRNA (*miRNA*) pathway has long been implicated in development and disease, including cellular stress. Recent studies have shown that an islet specific miRNA, *miR-375*, is crucial for the growth and function, notably insulin release, of pancreatic  $\beta$ -cells. The aim of the present study is to address the impact of *miR-375* on the pancreatic  $\beta$ -cell secretome from murine beta cell line MIN6, using the *Stable Isotope Labeling of Amino acids in Cell culture* (SILAC) based quantitative proteomic approach.

**Materials and methods:** MIN6 cells were labeled using SILAC method and *miR-375* and its targets were knocked-down systematically. 48hr later, secretion experiments were performed. The supernatant was concentrated and subjected to mass spectrometry to identify the respective secretome profiles.

**Results:** Using the SILAC based quantitative proteomic approach, we could identify over 600 proteins released, of which ~50 proteins that were regulated, in response to high glucose by the MIN6 beta cell line. Moreover, the secretome profiles following knockdown of *miR-375* and its target genes revealed differential regulation of many secreted proteins highlighting the complex regulatory role of this microRNA on the secretory machinery. Many of the proteins identified here, including the vitamin D binding protein, a gene previously correlated with the onset of Type I diabetes, have yet to be studied as a secreted factor from pancreas.

**Conclusion:** This study specifically addresses the role of *miR-375* in the systemic release of proteins from beta cells and provides insight into the future study of its direct targets and their role in exocytosis from this cell type.

## 214

**MicroRNA transfer as a new cell-to-cell communication mode between pancreatic beta cells**

C. Guay, V. Menoud, S. Gattesco, R. Regazzi;

Department of Cellular Biology and Morphology, University of Lausanne, Switzerland.

**Background:** MicroRNAs are important regulators of beta-cell functions. Recently, these small non-coding RNAs have been discovered to function not only inside the cells producing them, but also by their transfer in active form to recipient cells via microvesicles such as exosomes. This novel communication mode has been described in different cells of the immune system but remains poorly investigated in other biological systems. Two recent studies suggested that pancreatic beta-cell lines secrete microvesicles but their microRNA content was not explored. In the present study, we analyzed the microRNA content of microvesicles released by beta-cell lines and primary pancreatic islets and tested the biological relevance of beta-cell to beta-cell microRNA transfer.

**Material and methods:** MicroRNA-containing microvesicles released in the culture media of MIN6 or INS 832/13 beta-cell lines and of rat and human islets were isolated by ultracentrifugation. MIN6 cells were incubated in the presence of cytokines or palmitate to determine the impact of these pathophysiological conditions on microRNA release. Microvesicle preparations were first analyzed by microarray and by qRT-PCR to determine their microRNA content and were then used to study the impact of microRNA transfer on the function of recipient beta-cells.

**Results:** MicroRNAs were detected in the culture media of all beta-cell lines and primary islets tested. The microRNAs isolated from the culture media were fully protected against RNase treatment, suggesting that they are confined in microvesicle structures. Global profiling revealed that the microRNA content of microvesicles is not a mirror image of the cell content. Indeed, some microRNAs poorly expressed inside the cells were relatively abundant in microvesicles and vice-versa, indicating a preferential release of a subset of microRNAs. Moreover, incubation of MIN6 cells in the presence of cytokines or palmitate changed the level of several microRNAs released in the medium. We next investigated the dynamics of microRNA secretion and their transfer to recipient cells. Overexpression of miR-142-3p in MIN6 cells, a microRNA present at low level in beta-cells, resulted in a time-dependent release of this non-coding RNA in the culture media and its transfer into recipient MIN6 cells. Interestingly, incubation of untreated MIN6 cells in the presence of microRNA-containing microvesicles isolated from the culture media of MIN6 cells treated with cytokines led to an increase in cell death and a decrease in cell proliferation of recipient cells. In contrast, the microvesicles purified from the medium of untreated MIN6 cells did not affect the function of recipient cells.

**Conclusion:** Taken together, our results suggest that beta-cells are able to release microRNAs that can be transferred to neighboring beta-cells. Our observations indicate that a subset of microRNAs is preferentially selected for secretion. Exposure of donor cells to pathophysiological conditions associated with diabetes, such as treatment with cytokines or elevated fatty acid concentrations, modifies the release of microRNAs and affects the survival of recipient cells. More experiments will be needed to determine the precise contribution microRNA transfer to beta-cell dysfunction associated with diabetes. However, our results support the concept that microRNA transfer constitutes a novel cell-to-cell communication mechanism.

Supported by: FRSQ, SFD, CDA, FNS

## 215

**MicroRNA target sites as genetic tools to enhance promoter-reporter constructs for the purification of pancreatic progenitor cells from differentiated embryonic stem cells**

U. Diekmann, S. Lenzen, O. Naujok;

Institute of Clinical Biochemistry, Hannover Medical School, Germany.

**Background and aims:** Insulin-producing surrogate cells generated by in vitro differentiation from pluripotent cell sources (PSCs) such as ESCs or iPSCs hold great promises to overcome the limitations of transplantation therapies for T1DM. Differentiation of PSCs produces heterogeneous cell populations which must be further purified from unwanted cells and cells that resisted differentiation. In this study the specificity of tissue-specific promoter reporter constructs was increased utilizing the embryonic microRNA (miRNA) expression. This allowed the purification of pancreatic progenitors (PP) by fluorescence activated cell sorting (FACS).

**Materials and methods:** MiRNA expression in the mouse ESC lines ES-CCE and ES-D3 was analyzed by qPCR during the differentiation towards pancreatic endoderm. A lentiviral vector system that contained the tissue-specific Pdx1 promoter controlled the expression of the fluorescent reporter GFP2. This genetic cassette was further modified by insertion of a miRNA target site for the miRNA mir-294 (mirT294). Both cell lines were stably transduced and clones were clonally expanded. Subsequently the clones were subjected to differentiation with the small molecules IDE1 for 6 days and with ILV and FGF10 for the next 4 days. The cells were then analysed by flow cytometry and FACS-sorted cells were characterized by IF and qPCR. **Results:** The Pdx1 tissue-specific promoter construct showed slight background fluorescence after lentiviral transduction and clonal expansion. FACS-sorted clones at day 10 or 15 showed a 5.3-fold or 8.8-fold increased Pdx1 expression in the GFP+ population, respectively, compared to the GFP-cells. The embryonic transcription factor Oct3/4 was 26.9-fold (d10) or 16.3-fold (d15) increased in GFP+ cells. This indicates that the sorted population consists of PPs mixed with partially or fully undifferentiated cells. To reduce the background fluorescence in ESCs the miRNAs mir-294, -302a and -302d were assessed during the differentiation process and normalized to snoRNA-202. Mir-294 expression decreased gradually upon differentiation (d0  $1.70 \pm 0.18$ ; d10  $0.06 \pm 0.01$ ). In ESCs mir-302a and -302d expression was not detectable, peaked at day 6 and disappeared afterwards nearly completely (mir-302a d6  $4.43 \pm 0.65$ ; d10  $0.08 \pm 0.02$ ). The mirT294 was cloned into the lentiviral vector which decreased the basal GFP2 expression specifically in undifferentiated ESCs. This could be shown for the strong constitutive CMV- and for the Pdx1-promoter constructs. Upon differentiation of CMV clones the fluorescence became detectable again. Moreover, sorted cells with the CEmPdx1-promoter GFP2 mirT294 construct (containing a CMV enhancer element) showed an increased Pdx1 expression in the GFP+ population compared to the GFP- cells. The expression of Oct3/4 was highly decreased in GFP+ cells compared to the GFP- population.

**Conclusion:** Integration of the mirT294 site into the lentiviral vector reduced the artificial background fluorescence in ESCs and permitted the purification of PP cells. The mir-302 cluster is apparently important for the mesendodermal differentiation in the mouse system whereas in human ESCs this cluster is also essential for pluripotency. In summary, this study presents a promising technique to specifically knock-down the expression of a gene of interest eg. during developmental processes utilizing the cellular miRNA expression in mirT's.

Supported by: Rebirth

## 216

**Modifications of microRNAs expression affect beta cell function and survival before and after type 2 diabetes instauration**V. Nesca<sup>1</sup>, C. Guay<sup>1</sup>, C. Jacovetti<sup>1</sup>, V. Menoud<sup>1</sup>, R. Laybutt<sup>2</sup>, M. Prentki<sup>3</sup>, R. Regazzi<sup>1</sup>;<sup>1</sup>Department of Cell Biology and Morphology, Université de Lausanne, Switzerland, <sup>2</sup>Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, Australia, <sup>3</sup>Montreal Diabetes Research Center, University of Montreal, Canada.

**Background and aims:** Type 2 diabetes occurs when pancreatic beta-cells are unable to sustain insulin demand of the organism due to a decline in beta-cell function and an increase in beta-cell death. The mechanisms underlying dysfunction of beta-cells are complex and only partially characterized, since both genetic susceptibility and environmental factors are involved. In the present study, we investigated if a deregulation in the level of microRNAs, a class of non-coding RNAs known to be potent regulators of gene expression, could contribute to beta-cell dysfunction and death during the development of type 2 diabetes.

**Materials and methods:** MicroRNA expressions were analyzed in pancreatic islets of two models of type 2 diabetes, the db/db mice and the diet-induced obesity (DIO) mice, using microarray profiling. Functional effects resulting from expression changes were investigated in pancreatic beta-cell lines and in dispersed primary rat islet cells.

**Results:** We identified more than 60 microRNAs that are differently expressed in islets from db/db mice and/or from DIO mice. The most important changes were observed for miR-132, miR-199a-3p and miR-199a-5p which were up-regulated and miR-184, miR-203, miR-210 and miR-383, which were down-regulated in the islets of diabetic mice. The level of some of these microRNAs was already affected in islets of young pre-diabetic db/db mice, suggesting a progressive alteration during diabetes development. To investigate the role of these microRNAs in the regulation of beta-cell func-

tions and survival, we used oligonucleotide mimics or anti-miRs to up- or down-regulate, respectively, their level in MIN6B1 and in dispersed primary rat islet cells. Overexpression of miR-132 had a beneficial effect on beta-cells since it protected them from cytokine- and palmitate-induced cell apoptosis, promoted proliferation and glucose-induced insulin secretion. Down-regulation of miR-184 also increased proliferation, but did not improve beta-cell survival and insulin secretion. On the opposite, overexpression of miR-199a-3p or down-regulation of miR-203, miR-210 or miR-383 were detrimental to beta-cells since they induced apoptosis.

**Conclusion:** We observed changes in microRNAs expression in islets from type 2 diabetic animal models. Interestingly, some of the changes were already present before the development of the disease and resulted in improved beta-cell survival, proliferation and insulin secretion, suggesting that they may contribute to compensatory expansion of the functional beta-cell mass. In contrast, most of the changes observed in the islets of diabetic animals have detrimental impacts on beta-cells and are likely to contribute to beta-cell failure.

*Supported by: FNS, EFSD/MSD grant, FRSQ, CDA, SFD*

## OP 37 Metformin actions and benefits

217

**Effect of acute and short-term metformin treatment on 5'AMP-activated protein kinase regulation at rest and during exercise in human skeletal muscle and adipose tissue**

**J.M. Kristensen**<sup>1,2</sup>, C. Lillelund<sup>1</sup>, R. Kjøbsted<sup>1</sup>, J. Birk<sup>1</sup>, L. Nybo<sup>3</sup>, K. Mellberg<sup>4</sup>, A. Balendran<sup>4</sup>, E. Richter<sup>1</sup>, J. Wojtaszewski<sup>1</sup>

<sup>1</sup>Department of Exercise and Sport Sciences, Section of Human Physiology, Molecular Physiology Group, University of Copenhagen, Denmark,

<sup>2</sup>Department of Endocrinology, Section for Molecular Diabetes & Metabolism, Odense University Hospital and Institute of Clinical Research, University of Southern Denmark, Denmark, <sup>3</sup>Department of Exercise and Sport Sciences, Section of Human Physiology, Integrated Physiology Group, University of Copenhagen, Denmark, <sup>4</sup>AstraZeneca R&D, Mölndal, Sweden.

**Background and aims:** Metformin is a widely used drug for treatment of type II diabetic patients. Metformin works by suppressing hepatic glucose production, and also seems to improve insulin sensitivity by enhancing glucose uptake in skeletal muscle and adipose tissue. The molecular mechanism behind the effect of metformin is not fully elucidated, but activation of the 5'AMP-activated protein kinase (AMPK) has been observed in human skeletal muscle and adipose tissue after long-term treatment. Exercise is another potent stimulus for AMPK activation. We studied acute and short-term effects of metformin treatment on AMPK, in human skeletal muscle and adipose tissue at rest and during exercise.

**Materials and methods:** The study was conducted in a randomized blinded cross over design. Healthy moderately trained men were treated with metformin/placebo either once (3000 mg metformin) or for 4 days (increasing metformin dose from 1000-3000 mg daily) and conducted one bout of exercise after treatment. Blood samples and biopsies from the vastus lateralis muscle and from subcutaneous abdominal fat were obtained to study the effect of metformin treatment, acute exercise and the combination of these interventions. Differences between groups were considered statistically significant when  $P < 0.05$ .

**Results:** The metformin dosages elicited peak plasma and muscle metformin concentrations of 31  $\mu\text{M}$  and 11  $\mu\text{M}$ , respectively. The metformin treatment induced increased whole body stress indicated by significant increased plasma lactate (~60%), adrenaline (~25%), heart rate (~10%) and rate of perceived exertion during exercise. However, this was not reflected at the cellular level as the muscle metabolites lactate, creatine, phosphocreatine and glycogen were unaffected by metformin treatments both at rest and during exercise. Furthermore, neither of the treatments affected AMPK activity in resting skeletal muscle and adipose or potentiated exercise induced activation of AMPK in skeletal muscle.

**Conclusion:** We conclude that acute and short-term metformin treatment, despite eliciting high plasma metformin concentrations, does not affect AMPK at rest or during exercise in skeletal muscle and adipose tissue of healthy subjects.

*Clinical Trial Registration Number: H-KF-277313, 181777 and 18216*

*Supported by: DMRC, NNF, DDA, LE, UNIK*

218

**Metformin directly antagonizes the mitogenic effects of insulin, IGF-I and AspB10 (X10) insulin by regulating the cell cycle inhibitors cyclin G2 and p27kip**

**P. De Meyts**<sup>1</sup>, A.B. Jeppesen<sup>2</sup>, S. Winge<sup>1</sup>, A.M. Svendsen<sup>2</sup>, I.H. Lambert<sup>3</sup>, W. Sajid<sup>4</sup>

<sup>1</sup>Diabetes Biology and Hagedorn Research Institute, Novo Nordisk A/S, Gentofte, <sup>2</sup>Incretin Biology, Novo Nordisk A/S, Gentofte, <sup>3</sup>University of Copenhagen, <sup>4</sup>Diabetes Complications Biology, Novo Nordisk A/S, Gentofte, Denmark.

**Background and aims:** A debate is ongoing as to whether diabetes and some modalities of its treatment (e.g. with an insulin analogue) may increase the risk of cancer. In contrast, treatment with metformin has been proposed to have a beneficial effect on cancer risk. In previous work, we have shown that insulin, insulin-like growth factor-I (IGF-I) and a supermitogenic insulin analogue, X10, markedly downregulate the expression of mRNA and protein



for the cell cycle inhibitor cyclin G2 in several cell lines. Cyclin G2 is an atypical cyclin that, unlike other cyclins, blocks rather than stimulates cell cycle progression. Cyclin G2 has been found to be downregulated in a number of cancers. We showed that overexpression of cyclin G2 protected against the mitogenic effects of insulin, IGF-I and X10. We also found that insulin, IGF-I and X10 inhibit the protein level of another cell cycle inhibitor, p27<sup>Kip1</sup>. In the present work, we investigated whether metformin may counteract the mitogenic effects of insulin, IGF-I and X10 in wild-type L6 myoblasts (L6-WT, containing only IGF-I receptors (IGF-IR)) and L6 cells overexpressing the human insulin receptor (L6-hIR), and affect their downregulation of cyclin G2 and p27<sup>Kip1</sup>.

**Materials and methods:** Mitogenesis was measured by <sup>3</sup>H-thymidine incorporation into DNA. The mRNA levels of cyclin G2 and p27<sup>Kip1</sup> were measured by qRT-PCR, and the protein levels by Western blotting. The cells were starved of serum for 24 hours, and then exposed to insulin, IGF-I or X10 at various concentrations in the absence or presence of increasing concentrations of metformin for various periods of time.

**Results:** Insulin, IGF-I and X10 all markedly increased <sup>3</sup>H-thymidine incorporation into DNA in both L6-WT and L6 hIR cells. This effect was completely abolished at high concentrations of metformin, with an IC50 around 8 mM in L6-WT cells and 6.6 mM in L6-hIR cells. Metformin had no effect on the basal level of <sup>3</sup>H-thymidine incorporation into DNA. The antagonistic effect of metformin on mitogenesis induced by insulin, IGF-I and X10 was mimicked by the adenosine analogue 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an agonist of AMP kinase. As we showed previously, insulin, IGF-I and X10 downregulated the mRNA level for cyclin G2 as well as the protein level in a time- and concentration-dependent fashion in both cell lines, the effect being much more pronounced in the L6-hIR cells. Metformin significantly decreased the downregulating effect of the three ligands on cyclin G2. The three ligands also downregulated the protein (but not mRNA) levels of p27<sup>Kip1</sup>, an effect that was also significantly reduced by metformin.

**Conclusion:** We demonstrate for the first time that metformin directly antagonizes the mitogenic effects of insulin, IGF-I and X10 through both the insulin and IGF-I receptors in cell culture. This effect is mimicked by AICAR, suggesting an AMP kinase-mediated effect. This antagonism appears to be mediated through the regulation of mRNA and protein levels of cyclin G2, and protein level of p27<sup>Kip1</sup>, two inhibitors of the cell cycle and tumour suppressors. How this relates to putative cancer-protecting effects of metformin deserves further study.

## 219

**Metformin enhances secretion of GLP-1 but not GIP and improves postprandial glucose excursion in Japanese patients with type 2 diabetes**  
H. Kuwata<sup>1</sup>, D. Yabe<sup>1</sup>, K. Sugawara<sup>1</sup>, R. Usui<sup>1</sup>, K. Sugizaki<sup>1</sup>, Y. Kitamoto<sup>1</sup>, S. Fujiwara<sup>1</sup>, K. Watanabe<sup>1</sup>, T. Hyo<sup>1</sup>, T. Murakami<sup>2</sup>, T. Yamaguchi<sup>2</sup>, S. Shimizu<sup>2</sup>, H. Eto<sup>2</sup>, T. Kurose<sup>1</sup>, Y. Seino<sup>1</sup>

<sup>1</sup>Division of Diabetes, Clinical Nutrition and Endocrinology, <sup>2</sup>Division of Clinical Laboratory Science, Kansai Electric Power Hospital, Osaka, Japan.

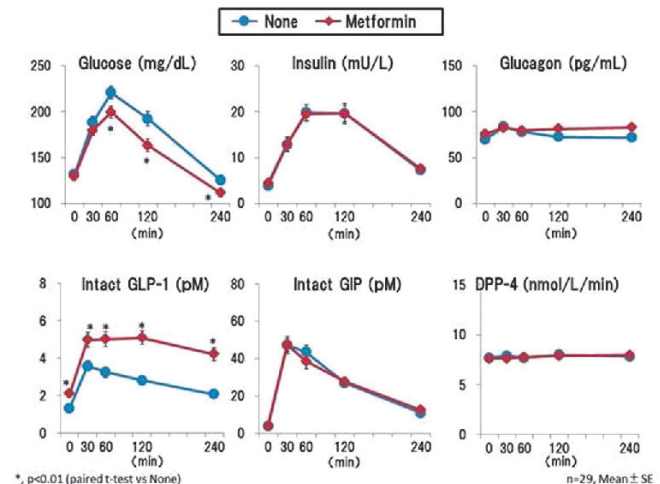
**Background and aims:** Recent studies have shown that metformin increases total and intact levels of glucagon-like protein-1 (GLP-1). However, little is known of the effects of metformin on secretion and metabolism of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) specifically in Asians. We evaluate here the effects of metformin on fasting and postprandial levels of GLP-1 and GIP in Japanese patients with type 2 diabetes.

**Materials and methods:** Twenty-nine untreated Japanese patients with type 2 diabetes [Age (year), 53.7±1.7; BMI (kg/m<sup>2</sup>), 27.6±1.1; HbA1c (NGSP%), 7.6±0.2] were subjected to meal tolerance test (MTT) using a Japanese standard breakfast (480 kcal; carbohydrate: protein: fat= 2.8:1:1) with or without administration of metformin (1,500mg/day). Blood samples were collected and total and intact GLP-1 and GIP levels were measured in addition to those of glucose, insulin, glucagon, and dipeptidyl peptidase-4 (DPP-4) activity.

**Results:** Metformin significantly lowered levels of plasma glucose at 60, 120 and 240 min after meal ingestion. The insulin/glucose ratio was elevated at 60, 120 and 240 min after meal ingestion, suggesting enhancement of postprandial insulin secretion relative to plasma glucose. Levels of fasting and postprandial GLP-1, both total and intact forms, were significantly elevated by metformin. In contrast, DPP-4 activity and total and intact GIP levels were unchanged throughout MTT.

**Conclusion:** In Japanese patients with type 2 diabetes, metformin improves the postprandial glucose excursion partly through elevation of intact GLP-1, but the drug does not affect GIP. Elevation of intact GLP-1 in these patients

is therefore likely due to enhanced insulin secretion rather than to inhibition of its degradation by DPP-4.



## 220

**Metformin improves intestinal L cell survival in vitro and in vivo**  
R. Jakubov<sup>1</sup>, P.L. Brubaker<sup>2</sup>, L.M. Lauffer<sup>3</sup>

<sup>1</sup>Internal Medicine II, Klinikum rechts der Isar, Munich, Germany,

<sup>2</sup>Physiology, University of Toronto, Canada, <sup>3</sup>Internal Medicine IV, Klinikum der Universität München, Munich, Germany.

**Background and aims:** Glucagon-like peptide-1 (GLP-1) is an insulinotropic hormone released from the intestinal L cell following stimulation by nutrients and other secretagogues. Hence, GLP-1 analogs and degradation inhibitors are now used in the therapy of type 2 diabetes. As recently reported, metformin, an AMP-protein kinase (AMPK) activator and a well established anti-diabetic drug, causes increased GLP-1 levels in vivo. Although the exact mechanism remains incompletely defined, direct stimulation of the intestinal L cells with metformin in vitro did not increase GLP-1 secretion. We hypothesized that the increased GLP-1 levels might be associated with enhanced L cell proliferation and decreased L cell apoptosis.

**Materials and methods:** To assess L cell survival, the mouse GLUTag L cell line was treated for 24 hr with metformin (150mM) or the AMPK-activator AICAR (1mM), followed by induction of apoptosis with cytokines or staurosporine, and immunoblot for cleaved Caspase-3, a marker of apoptosis. L cell proliferation following the same treatment (metformin or AICAR) was assessed by <sup>3</sup>H-thymidine incorporation. The effect of metformin in vitro was examined in rats treated neonatally with streptozotocin, a model of type 2 diabetes. The rats were maintained on a standard laboratory diet for 8 weeks, followed by 4 weeks treatment with metformin (300 mg/kg BW/day, supplied in water) with and without sitagliptin (2.8 g/kg BW, supplied in food) to further increase GLP-1 levels. The metformin and sitagliptin dose was adjusted daily according to daily measurements of drinking volume and bodyweight. The terminal 2 cm of the ileum was then extracted and cross-sections of the terminal ileum (200µm apart) were stained with a GLP-1 specific antibody, to visualize the L cells. For every rat at least 4 intestinal cross-sections were analyzed for absolute L cell counts.

**Results:** Incubation of GLUTag cells with metformin (150mM) or AICAR (1mM) decreased L-cell apoptosis by 33±4% (p<0.01) and 8±1% (p<0.01), respectively. While the same concentration of metformin did not affect proliferation of the GLUTag cells, AICAR increased proliferation by 74±7% compared to control (p<0.001). The rats treated with metformin showed a significant increase in the L cell counts (1.4±0.1 fold increase vs. control; n=4-6 rats; p<0.05). The addition of sitagliptin to metformin did not modulate the response to metformin (1.5±0.1 fold increase vs. control; n=4 rats; p<0.05).

**Conclusion:** Although metformin increases GLP-1 levels in patients, metformin does not directly stimulate GLP-1 release from intestinal L cell lines. As shown in our study, metformin improves survival of the L cells by decreasing apoptosis, potentially by modulating the AMPK signaling pathway. Additionally, metformin treatment in vivo increased the L cell counts, independent of sitagliptin, indicating a GLP-1-independent mechanism of the effect. Nonetheless, the mechanism of action of metformin to increase GLP-1 levels in humans in vivo needs further exploration. Taken together, these findings

provide further support for the effects of metformin to enhance GLP-1 levels, leading to improved control of type 2 diabetes in patients.

Supported by: EFSD/Lilly fellowship, Merck Investigator-Initiated Study Program

## OP 38 Hypoglycaemia

### 221

#### The effect of mild and severe hypoglycaemia and hypoglycaemia awareness on 12-year all-cause mortality in type 1 diabetes

A.-S. Sejling<sup>1</sup>, B. Thorsteinsson<sup>1,2</sup>, U. Pedersen-Bjergaard<sup>1</sup>;

<sup>1</sup>Department of cardiology, nephrology and endocrinology, Hillerød Hospital, <sup>2</sup>Faculty of Health Sciences, University of Copenhagen, Denmark.

**Background and aims:** Severe hypoglycaemia is reportedly associated with excess all-cause mortality and other adverse clinical outcomes in type 2 diabetes. It is presently unknown whether hypoglycaemia is a marker of underlying co-morbidities rather than a direct cause of death. We studied if an association exists between occurrence of episodes of mild (MH) and severe hypoglycaemia (SH) and long-term all-cause mortality in type 1 diabetes. Similar analyses were conducted for hypoglycaemia awareness since impaired awareness predisposes to both silent and severe hypoglycaemia.

**Materials and methods:** Twelve-year all-cause mortality was recorded in a cohort of 265 patients with type 1 diabetes (60% men; age 45±13 years (mean±SD) and duration of diabetes 21±12 years at entry). At baseline patients were asked about life-time number of SH and number of SH during the last two years, and their hypoglycaemia awareness class (44% aware, 44% impaired, and 12% unaware) was characterized. The patients were subsequently followed monthly for one year. SH was defined by third party assistance and was recorded and validated in telephone interviews within 24 hours in the prospective part of the study. MH was defined as episodes with symptoms familiar to the patient as hypoglycaemia and managed solely by the patient. These episodes were reported for the week preceding the return of the monthly questionnaires during the prospective part of the study.

**Results:** The 12-year all-cause mortality was 15% (n=39). SH and all-cause mortality were not associated, neither when retrospectively recorded (life-time number of episodes divided in 0-1 (46%), 2-5 (23%), 6-10 (10%) and >10 episodes (21%); p=0.09); the last two years before entry: 0-1 (69%), 2-5 (20%) and >5 episodes (11%); p=0.49), nor when prospectively recorded for one year after entry (0-1 (85%), 2-5 (10%) and > 5 episodes (5%); p=0.34). Occurrence of SH in the two last years before baseline (no=55%, yes=45%) and all-cause mortality were not associated (p=0.20). No associations between all-cause mortality and MH at baseline (0-1 (45%), 2 (29%) and >2 episodes per week (26%); p=0.22) or in the first year after entry (0-1 (37%), 2 (30%) and >2 episodes per week (33%); p=0.16) were observed. Finally, no differences existed in all-cause mortality between awareness classes (p=0.45). All-cause mortality was associated with long duration of diabetes (p=0.002), high age (p<0.001), and male gender (p=0.04), but not with HbA1c at entry (<7% (6%), 7-8.5% (46%) and >8.5% (48%); p=0.29).

**Conclusion:** We did not observe any association between long-term all-cause mortality and the occurrence of mild and severe hypoglycaemic episodes in type 1 diabetes. Likewise, mortality was not associated with class of hypoglycaemia awareness.

### 222

#### GAPP2™: Global survey finds three quarters of patients experience hypoglycaemia on insulin analogue causing dose irregularities and increased blood glucose monitoring

A. Tahrani<sup>1</sup>, A.H. Barnett<sup>2</sup>, M. Brod<sup>3</sup>, A. Rana<sup>4</sup>, M. Peyrot<sup>5</sup>;

<sup>1</sup>University of Birmingham, UK, <sup>2</sup>Heart of England NHS Foundation Trust and University of Birmingham, UK, <sup>3</sup>The Brod Group, Mill Valley, USA,

<sup>4</sup>Novo Nordisk, Copenhagen, Denmark, <sup>5</sup>Loyola University, Baltimore, USA.

**Background and aims:** Hypoglycaemic events are a major challenge in glycaemic control of type 2 diabetes (T2DM) with insulin and have a significant impact on morbidity, mortality and quality of life. A large global survey (GAPP2™) was conducted with T2DM patients taking insulin, and healthcare professionals (HCPs) (primary care, diabetes specialists and diabetes nurses/educators) to examine the frequency of self-treated hypoglycaemia (hypo) and assess and compare how patients and HCPs adjust insulin treatment in response to these events.

**Materials and methods:** A survey was performed in 6 countries in Europe, Asia and North America. 1653 HCPs managing T2DM and 3587 T2DM patients on insulin were recruited from online research panels with over 6.5 million members. The HCP and patient surveys included 50-70 questions examining dosing irregularities and hypos in T2DM managed with insulin.

**Results:** Data are presented from 3042 T2DM patients on insulin analogue (IA) and 1653 HCPs on their IA T2DM patients. 80% of patients reported having a hypo, with 76% of the most recent events diurnal and 24% nocturnal. 36% of patients had had a hypo in the last 30 days and the mean number of events reported by these patients was  $3.1 \pm 0.09$  per month ( $2.3 \pm 0.07$  diurnal and  $0.8 \pm 0.05$  nocturnal). 46% of patients had increased their blood glucose monitoring and more than 10% had altered their basal insulin (BI) regimen in response to a hypo. For the most recent hypo, patients reported missing (5%), reducing (7%) or mis-timing (3%) a mean of  $1.8 \pm 0.17$ ,  $4.2 \pm 2.08$ , and  $2.6 \pm 0.71$  BI doses. Patients reported that in order to reduce their risk of nocturnal hypos they had intentionally let their blood glucose go high (14%) or had not taken their insulin as prescribed (16%). Hypos had an impact on resource utilization as well as on disease management. 52% of HCPs reported being contacted at least once a month by IA patients after hypos and 82% reported that they consider hypo risk when choosing the type of insulin they initiate. 57% reported starting patients on a lower insulin dose than recommended due to risk of hypos. HCPs reported that on most occasions they advise patients who have a number of hypos to increase their blood glucose monitoring (35%), temporarily reduce (19%), reduce long-term (16%) or split (3%) their BI dose.

**Conclusion:** Hypos are extremely common for T2DM patients using IA and have a major impact on the way patients and HCPs manage diabetes. In response to hypos many patients increase blood glucose monitoring and some intentionally keep their blood glucose at a higher than appropriate level or adjust their BI dose to avoid these events. Many of the patient responses to hypos are inappropriate and suggest a need for further education or more HCP support.

#### Patient regimen modification in response to self-treated hypoglycaemia (hypo)

	Hypo patient response - ever	Hypo patient response - most recent event
Patients ever experiencing a mild hypo	2348	2348
Miss a basal dose	8%	5%
Mean number of doses missed		$1.8 \pm 0.17$
Basal dose taken >2 hrs earlier or >2 hrs later than prescribed	5%	3%
Mean number of doses mis-timed		$2.6 \pm 0.71$
Reduced a dose of basal	12%	7%
Mean number of doses reduced		$4.2 \pm 2.08$
Increased level of blood glucose monitoring	46%	40%
Mean length of time of increased monitoring (h)		$32.2 \pm 3.05$

Supported by: The GAPP2 surveys were supported by a grant from Novo Nordisk

## 223

### Effect of hypoglycaemia on ECG and EEG in type 1 diabetes

C.B. Juhl<sup>1,2</sup>, A. Larsen<sup>1</sup>, K. Højlund<sup>3</sup>, R. Madsen<sup>1</sup>;

<sup>1</sup>Hypo-Safe, Lyngby, <sup>2</sup>Department of Endocrinology, Sydvestjysk Sygehus, Esbjerg, <sup>3</sup>Department of Endocrinology, Odense University Hospital, Denmark.

**Background and aims:** Tight glycaemic control in type 1 diabetes may only be accomplished if severe hypoglycaemia can be prevented. Bio-sensor alarms based on the body's reactions to hypoglycaemia have previously been studied. Such alarms were based on different parameters such as skin conductance, electrocardiography (ECG) or electroencephalography (EEG). We have recently proposed an alarm based on continuous EEG monitoring and real-time data processing. In the present study we analysed three lead ECG and single channel EEG in type 1 diabetes patients exposed to insulin induced hypoglycaemia.

**Materials and methods:** From a group of fifteen patients exposed to insulin induced hypoglycaemia nine patients were included in the present analyses selected on the basis of the ECG and EEG quality. Glucose was gradually lowered by graded insulin infusion aiming a steady fall of glucose of 1 mmol/l/15 minutes and continued until the study subjects were obviously affected by

the low glucose. ECG and EEG was analysed post-hoc. Heart rate (HR), QTc interval (Bassett's method) and TpTec (time from top of T-wave to end of T-wave corrected for heart beat interval) were automatically calculated from the ECG recording. Three EEG features were extracted based on the spectral content of the signals in the delta band (1-4 Hz), the theta band (4-8 Hz), and the alpha band (8-13 Hz). Power estimates were computed continuously for 4 second epochs with 75% overlap between epochs. Both ECG and EEG features were thus calculated continuously allowing detection of the time of significant changes. The predefined variables were presence of ECG and EEG changes and time between detection of these changes and symptoms of severe hypoglycaemia.

#### Results:

Feature	True positive	False positive	Detection range (min)
<i>ECG features</i>			
QTc	6/9	1/9	0-26
TpTec	5/9	2/9	0-23
HR	5/9	3/9	2-16
<i>EEG features</i>			
EEG delta power	3/9	0/9	8-13
EEG theta power	8/9	0/9	4-24
EEG alpha power	4/9	0/9	7-20

The table shows detection rate and timing of hypoglycaemia associated ECG and EEG changes following graded insulin induced hypoglycaemia (N=9)

**Conclusion:** Severe hypoglycaemia is preceded by changes in both ECG and EEG features in the majority of the cases. EEG features appeared before severe hypoglycaemia in all cases and EEG theta power may be superior with respect to timing, sensitivity and specificity of severe hypoglycaemia detection. A multi-parameter algorithm which combines data from different biosensors might be considered.

Clinical Trial Registration Number: NCT00810420

## 224

### Liver glycogen loading augments the counterregulatory response to hypoglycaemia

J.J. Winnick<sup>1</sup>, G. Kraft<sup>1</sup>, W. Snead<sup>2</sup>, B. Farmer<sup>1</sup>, A.D. Cherrington<sup>1</sup>;

<sup>1</sup>Molecular Physiology & Biophysics, <sup>2</sup>Medicine, Vanderbilt University, Nashville, USA.

**Background and aims:** Latrogenic hypoglycemia is a persistent barrier to the safe, effective treatment of insulin-requiring people with type 1 and type 2 diabetes. During hypoglycemia, glucagon, epinephrine and cortisol are the primary counterregulatory hormones secreted into the blood and act to increase hepatic glucose output and reduce muscle glucose utilization, thereby restoring blood glucose homeostasis. The most readily available hepatic substrate to combat hypoglycemia is glycogen. Therefore, the purpose of this study was to determine whether hepatic glycogen content influences the response to a hypoglycemic challenge.

**Materials and methods:** During the first 4h of each study dogs received somatostatin and basal amounts of intraportal insulin and glucagon. Arterial blood glucose was doubled by glucose infusion into a peripheral vein and either saline (SAL; n=10) or fructose (FRU; 5.5  $\mu$ mol/kg/min; n=6) was infused into the hepatic portal vein, with the latter significantly stimulating hepatic glucose uptake and glycogen deposition as we have previously described. This glycogen loading period was followed by 2h of continued hyperglycemic/normoinsulinemia and the intraportal infusion of SAL or FRU was discontinued. During the final 2h period, hypoglycemia was induced in all animals using a 16x basal intraportal infusion of insulin. Animals from SAL were divided into two groups; one in which the endogenous counterregulatory hormone responses were allowed (SAL-ENDO; n=6) and a second group in which counterregulatory hormone responses were matched (SAL-MATCH; n=4) to those of FRU. Liver glycogen content at the outset of the hypoglycemic challenge was ~54 mg/g in SAL and ~82 mg/g in FRU.

**Results:** During the final hour of the 2h hypoglycemia period, arterial plasma glucose levels averaged  $2.6 \pm 0.1$  mmol in all three groups and the hepatic sinusoidal insulin levels were also similar ( $2514 \pm 102$ ,  $2340 \pm 186$  and  $2802 \pm 342$  pmol/l in SAL-ENDO, FRU and SAL-MATCH, respectively). In the glycogen loaded liver, net hepatic glucose output was greater during hypoglycemia ( $28 \pm 3$  and  $9 \pm 1$   $\mu$ mol/kg/min in FRU and SAL-ENDO, respectively;  $p < 0.001$ )



as were sinusoidal glucagon ( $108 \pm 25$  and  $65 \pm 7$  ng/l, respectively;  $p=0.06$ ) and arterial epinephrine ( $1791 \pm 356$  and  $955 \pm 225$  ng/l, respectively;  $p<0.05$ ) levels. When the glucagon ( $133 \pm 14$  ng/l) and epinephrine ( $1905 \pm 419$  ng/l) levels were matched in SAL-MATCH to those of FRU, net hepatic glucose output increased to a level ( $25 \pm 3$   $\mu\text{mol/kg/min}$ ) similar to that seen in FRU. Cortisol levels rose significantly in all groups, but did not differ among them.

**Conclusion:** Loading the liver with glycogen increases hepatic glucose output in response to hypoglycemia. Furthermore, this increase is mediated by an increase in the secretion of the counterregulatory hormones glucagon and epinephrine, suggesting that the brain may receive sensory feedback from the liver regarding the glucose stores that are available for mobilization. Thus, therapies that normalize liver glycogen content may be beneficial in preventing iatrogenic hypoglycemia in insulin-requiring humans with diabetes.

*Supported by: NIH-NIDDK*

## OP 39 Diabetes education and its clinical impact

225

### Does duration of type 1 diabetes affect the outcomes of structured education?

J. Elliott<sup>1</sup>, S.R. Heller<sup>1</sup>, H.E. Hopkinson<sup>2</sup>, P. Mansell<sup>3</sup>;

<sup>1</sup>Department of Human Metabolism, University of Sheffield, <sup>2</sup>Department of Diabetes and Endocrinology, Victoria Infirmary, Glasgow, <sup>3</sup>Department of Diabetes and Endocrinology, University of Nottingham, UK.

**Background and aims:** One belief held by some physicians is that structured education in patients with Type 1 diabetes (T1DM) is best delivered within a few years of diagnosis, before inappropriate self-management strategies become ingrained. In this study we examined whether the duration of diabetes does influence the effectiveness of a structured education course in patients with T1DM, both in terms of biomedical and psychosocial parameters.

**Materials and methods:** The study was undertaken using the Dose Adjustment For Normal Eating (DAFNE) research database, which recruits patients undertaking DAFNE training as part of their normal clinical care, from 10 English secondary care diabetes centres. Ethics approval for this research database was approved by the Trent Research Ethics Committee, Rec No. 08/H0405/48. The first 479 patients with one year follow-up data were analysed (52% female, baseline mean $\pm$ SD: age  $41.2 \pm 13.9$  years, duration of diabetes  $17.3 \pm 15.0$  years). Biomedical and psychosocial questionnaire data were collected prior to, and 12 months after patients completed DAFNE education. Statistical significance was determined using paired Student's t-tests for continuous data and Chi-squared tests for categorical data, where a p value of  $<0.05$  was considered to be statistically significant. Correlations were examined utilising linear regression analyses.

**Results:** At 1 year follow-up HbA1c had decreased from  $8.7 \pm 1.5\%$  ( $72 \pm 17$  mmol/l) to  $8.5 \pm 1.5\%$  ( $70 \pm 17$  mmol/l) ( $p=0.002$ ). In those 381 patients with a baseline HbA1c of  $>7.5\%$  the decrease was  $9.2 \pm 1.3\%$  ( $77 \pm 14$  mmol/l) to  $8.9 \pm 1.5\%$  ( $73 \pm 16$  mmol/l) ( $p<0.001$ ). The number of episodes of severe hypoglycaemia in the preceding 12 months was greatly reduced from 393 to 99 ( $p<0.001$ ), and the number of patients experiencing one or more episodes had decreased from 101 to 42 ( $p<0.001$ ). Similar reductions were seen with diabetic ketoacidosis, the number of episodes decreased from 63 to 19 ( $p<0.001$ ) and the number of patients experiencing these episodes had decreased from 45 to 12 ( $p<0.001$ ). Quality of life improved as evaluated by the Problem Areas in Diabetes questionnaire (a measure of diabetes distress)  $29.1 \pm 20.2$  to  $21.2 \pm 17.6$  ( $p<0.001$ ), and Euro Qol-5D state of health  $71.3 \pm 17.6$  to  $74.5 \pm 18.2$  ( $p=0.001$ ). The presence of anxiety and/or depression was assessed using the Hospital Anxiety and Depression questionnaire. The number of patients with a probable anxiety or depression disorder decreased from 64 to 41 ( $p<0.001$ ) and from 27 to 21 ( $p<0.001$ ) respectively, furthermore the mean anxiety and depression scores decreased from  $4.8 \pm 4.6$  to  $4.2 \pm 4.4$  ( $p<0.001$ ) and  $3.0 \pm 3.6$  to  $2.6 \pm 2.5$  ( $p<0.001$ ) respectively. There was no correlation between the changes in any of the above biomedical or psychosocial measures following DAFNE education, and the duration of diabetes ( $R^2 \leq 0.01$ ).

**Conclusion:** Whilst improvement in HbA1c was modest in this cohort, this study shows that structured education for patients with Type 1 diabetes is equally effective irrespective of the duration of diabetes. The biomedical and psychosocial benefits of DAFNE skills training are independent of the duration of diabetes. Therefore a long duration of diabetes should not be a barrier to patient participation.

*Supported by: NIHR UK under the Programme for Applied Research*

226

### Effect of a 5 days intervention programme for people with type 1 diabetes and severe hypoglycaemia

M. Glindorf, M. Wittrup;

Steno Patient Care Center, Steno Diabetes Center, Gentofte, Denmark.

**Background and aims:** The risk of developing late diabetic complications in type 1 diabetes is reduced by tight glycaemic control, but tight glycaemic control increases the frequency of hypoglycaemia. With increasing duration of diabetes severe hypoglycaemia (SH) defined as an episode requiring assistance from another person, becomes a significant problem, as many patients experience unawareness, and this gives rise to much concern. Since 1999 the

Clinic has conducted a 5 days inpatient Hypo-course for patients with type 1 diabetes prone to severe and frequent hypoglycaemia. An evaluation is now made to investigate if the patients experiences new acting competences to prevent and to handle SH. The aim of the course is to avoid or decrease the number of SH without increasing HbA1c, that patients regain awareness, decrease anxiety and improve behaviour in relation to prevent SH. Here we report results in relation to the last 24 participants.

**Materials and methods:** 15 Hypo-courses, each course includes 8 participants, with a total of 124 participants. The course is a 5 day inpatient course run by a multidisciplinary team, where communication in a narrative approach is fundamental and the philosophy is empowerment. The course contains knowledge in a daily life context about the relation between insulin, injection technique, monitoring blood glucose, awareness training, food and physical activity, alcohol, hypoglycaemia and unawareness. A daily activity-programme includes physical activity and exchange of experience between attendants who are also encouraged to experimental activities. A session with cohabitants includes sharing of feelings, concerns and involvement living with a person with SH. The present evaluation is based on both retrospective and prospective results and analysed in a mixed method design, with both quantitative and qualitative data. This presentation focuses on the result from the last three courses. 24 participants have answered 3 questionnaires: PAID, The HFS, WHO-5 before the course and 6 month later, and 1 questionnaire at the end of the course asking participants if they have got useful knowledge. We also measured HbA1c, self reported SH and change in awareness.

**Results:** 24 patients aged  $49.0 \pm 20$  years, 41.6 % female, A1C  $65.54 \pm 22.46$  mmol/mol, disease duration  $26.7 \pm 19$  years, microvascular complication 50 %, multiple complication 4.1 %, 91.6% with multiple insulin analog injections. At baseline the incidence of SH the last 2 years are > 21 (20.8 %) 11–20 (16.6 %) 6–10 (4.1 %) 1–5 (54.1 %). After 6 month average HbA1c was unchanged, patients improved hypoglycaemia awareness and 3 regained awareness, 91.6 % had no new SH. At the end of the course 91.6 % understand on an individual level how they can prevent SH based knowledge gained at the course. The HFS score improved significant ( $P = 0.004$ ), PAID score improved significant ( $P = 0.001$ ), WHO-5 no significant changes ( $P = 0.167$ ).

**Conclusion:** SH is a major problem for some patients with type-1 diabetes. The present Hypo-course concept based on empowerment and a narrative approach improves behaviour in relation to avoid SH without increasing HbA1c and results in a substantial decrease in the number of new episodes of SH, it decreases anxiety, improves behaviour in relation to avoid SH, and some patients regains or improves awareness.

Supported by: Due-Christensen, M

## 227

### The maximum exercise is safe for the well-educated patients with type 1 diabetes

P. Niedzwiecki, A. Gawrecki, D. Naskret, A. Duda-Sobczak, S. Karbowska, B. Wierusz-Wysocka, D. Zozulinska-Ziolkiewicz, Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poland.

**Background and aims:** According to the Diabetes Poland recommendations intense, short exercise (> 90% VO<sub>2</sub>max) can lead to hyperglycemia and metabolic acidosis. Therefore aerobic exercise is recommended in patients with type 1 diabetes. The aim of the study was to assess the risk of decompensation of type 1 diabetes during maximal exercise.

**Materials and methods:** 10 well educated men, treated with personal insulin pump, aged  $23.0 \pm 5.6$  years, type 1 diabetes duration:  $11.1 \pm 3.7$  years, HbA1c  $7.0 \pm 0.2$  % (53 mmol/mol) were involved. Patients begun the exercise on a treadmill in 90th minute after ingestion of a standardized meal containing 90g of carbohydrates. Physical exercise of progressively increasing intensity was continued until the subjective sensation of fatigue assessed in 10 point Borg scale. The concentrations of lactic acid in serum and ketone bodies in the capillary blood were assessed before exercise, immediately after its completion, and after 6 hours. Glycaemia was measured using glucose meter and continuous glucose monitoring system (CGMS) before, during exercise and for 6 hours after its completion. Exercise test was performed twice during two different days.

**Results:** Serum concentration of lactic acid was: before exercise:  $1.57 \pm 0.39$  mmol/l, immediately after exercise:  $5.98 \pm 3.8$  mmol/l ( $p < 0.0001$ ; immediately after exercise vs before) and 6 hours after exercise:  $0.97 \pm 0.46$  mmol/l ( $p < 0.0005$ ; 6 hours after exercise vs before). No increase in ketone bodies concentration > 0.5 mmol/l was observed during the study. The distance achieved during the exercise was:  $3994 \pm 1593$  meters. Exercise time was:  $28.3 \pm 8.1$

minutes. Glycaemia measured on glucose meter before ingestion of standardized meal was:  $8.6 \pm 2.4$  mmol/l, before exercise (BE) was:  $10.9 \pm 2.0$  mmol/l, at the end of exercise (EE):  $7.4 \pm 2.5$  mmol/l ( $p < 0.0001$ ; BE vs EE), at 6 hour of observation(AE):  $10.5 \pm 3.7$  mmol/l ( $p = 0.62$ ; BE vs AE). Average glycaemia measured by CGMS before exercise (BE) was:  $9.8 \pm 2.2$  mmol/l, during exercise (DE):  $10.9 \pm 3.0$  mmol/l ( $p = 0.05$ ; BE vs DE), during 6 hours of observation (AE):  $9.9 \pm 2.6$  mmol/l ( $p = 0.77$ ; BE vs AE). There were no episodes of hypoglycaemia.

**Conclusion:** In the studied group the maximal exercise did not cause decompensation of type 1 diabetes.

## 228

### Health-related quality of life associated with daytime and nocturnal hypoglycaemic events: a time trade-off survey

J. Gungaard<sup>1</sup>, S.B. Harris<sup>2</sup>, M. Evans<sup>3</sup>, K. Khunti<sup>4</sup>, M. Mamdani<sup>5</sup>, C.B. Galbo-Jørgensen<sup>6</sup>, M. Bøgelund<sup>6</sup>,

<sup>1</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>2</sup>Centre for Studies in Family Medicine, University of Western Ontario, London, Canada, <sup>3</sup>Llandough Hospital, Cardiff, UK, <sup>4</sup>University of Leicester, UK, <sup>5</sup>Department of Health Policy, Management, and Evaluation (Faculty of Medicine) and Leslie Dan Faculty, University of Toronto, Canada, <sup>6</sup>Incentive Partners, Holte, Denmark.

**Background and aims:** Hypoglycaemic events (hypos) can affect health-related quality of life (HRQoL) via acute symptoms, altered behavior and fear of future hypos. Nocturnal hypos, in particular, are unpredictable and difficult to avoid, and may affect HRQoL more than daytime hypos. We examined if HRQoL differs between daytime and nocturnal, severe and non-severe hypos, using time trade-off (TTO) methods.

**Materials and methods:** HRQoL was quantified via an online TTO survey with 10,087 respondents from the general population in the UK, USA, Canada, Germany and Sweden. Hypos were defined based on symptoms, and were classified as severe if third-party assistance was required. Descriptions of hypo health states were derived from a survey of 247 UK patients with diabetes. HRQoL was measured on a utility scale with 0 equating to dead and 1 perfect health. Respondents traded-off length of life for improving health states and evaluated health states of well-controlled diabetes and diabetes with non-severe/severe and daytime/nocturnal hypos.

**Results:** Table 1 shows the results. Non-severe nocturnal hypos were associated with 0.0026 (95% CI 0.0016; 0.0034) higher disutility compared with non-severe daytime hypos (equivalent to a 63.4% increase in negative impact); severe nocturnal hypos were associated with 0.0057 (95% CI -0.0001; 0.0114) higher disutility compared with severe daytime hypos (not statistically significant).

**Conclusion:** The study estimates the disutility associated with diabetes, uniquely illustrating the detrimental effect of hypos on HRQoL and in particular demonstrating the adverse effect of nocturnal hypos on HRQoL compared with daytime hypos. Indeed, the impact of nocturnal hypos have, in the past, been underappreciated by both clinicians and patients. These results highlight the clinical imperative of avoiding hypos, especially nocturnal hypos, to improve HRQoL at the patient level.

Table 1. Utility estimates from the time trade-off survey

Type of hypoglycaemia	n	Average utility	Standard error	95% CI lower	95% CI upper
Diabetes with no hypoglycaemia	7642	0.8440	0.0022	0.8395	0.8484
Non-severe daytime event	7435	-0.0041	0.0003	-0.0035	-0.0047
Non-severe nocturnal event	7347	-0.0067	0.0004	-0.0059	-0.0074
Severe daytime event	3603	-0.0565	0.0021	-0.0527	-0.0606
Severe nocturnal event	3647	-0.0622	0.0021	-0.0582	-0.0663

Supported by: Novo Nordisk

## OP 40 Glucose fluctuations and cardiac complications

229

### Prognostic value of admission glycaemic excursions in elderly patients with acute coronary syndrome

S. Mi<sup>1</sup>, G. Su<sup>1</sup>, Z. Li<sup>2</sup>, H. Yang<sup>1</sup>, H. Zheng<sup>1</sup>, H. Tao<sup>3</sup>, Y. Zhou<sup>1</sup>, L. Tian<sup>1</sup>;  
<sup>1</sup>Beijing An Zhen Hospital of Capital Medical University, <sup>2</sup>Emergency center, Beijing An Zhen Hospital of Capital Medical University, <sup>3</sup>Endocrinology department, Beijing An Zhen Hospital of Capital Medical University, China.

**Background and aims:** Acute phase hyperglycaemia has been associated with increased mortality in patients with acute coronary syndrome (ACS). However, the predictive value of admission glycaemic excursions for adverse outcome in elderly ACS patients is unknown. This study is to investigate the prognostic value of admission glycaemic variability for one-year major adverse cardiac event (MACE) in elderly patients with ACS.

**Materials and methods:** 186 elderly ACS patients' clinical data were collected and the GRACE risk score were calculated as admission. The fluctuations of glucose levels in patients were measured by a continuous glucose monitoring system (CGMS) for 72 hours. The occurrence of MACE in patients was documented during one year follow-up. All participants were grouped into tertiles of mean amplitude of glycaemic excursions (MAGE), absolute means of daily differences (MODD) and postprandial glucose excursion (PPGE) to compare the baseline data, the level of GRACE risk score and the incidence of MACE among the subgroups. A multivariate Logistic stepwise regression model was made to explore the independent contribution to cardiovascular outcomes.

**Results:** In all patients, a higher MAGE, MODD or PPGE level was associated with advanced age, the higher levels of heart rats, admission blood glucose and hemoglobin A<sub>1c</sub>, the lower level of left ventricular ejection fraction value, and a higher GRACE risk score and higher incidence of MACE. The same results were also found in elderly ACS patients with and without diabetes history. Levels of MAGE (4.07±1.37 mmol/L vs. 2.77±1.37 mmol/L,  $p<0.001$ ), MODD (2.66±1.01mmol/L vs. 1.62±0.76mmol/L,  $p<0.001$ ) and PPGE (6.06±2.61 mmol/L vs. 3.47±2.13 mmol/L,  $p<0.001$ ) were significantly higher in patients with MACE than in patients without MACE. Multivariate analysis indicated that age, previous CAD history, high-sensitive C reactive protein, admission blood glucose, eGFR, MAGE and MODD were independent determinants for MACE. After adjusting for GRACE risk score, diabetes history, hypoglycemic therapy and other confounders, MAGE ( $p=0.003$ ) and MODD ( $p=0.052$ ) levels retained a significant independent association with occurrence of MACE.

**Conclusion:** The admission intraday and interday glycaemic variability may be associated with the outcome of elderly patients with ACS. Acute glycaemic excursions, as one of components of the dysglycaemia, should not be neglected in ACS patients.

Supported by: Beijing Health Special Foundation

230

### Glycaemic variability and ventricular cardiac arrhythmias in type 2 diabetic patients with chronic heart failure

I. Pochinka, L. Strongin, J. Struchkova;  
 The Nizhny Novgorod State Medical Academy, Russian Federation.

**Background and aims:** Prevalence of chronic heart failure in population with diabetes is wide. Ventricular arrhythmias seem one of the factors that determine the prognosis of heart failure. It has to be checked whether glycaemic variability has an influence on ventricular cardiac arrhythmias in type 2 diabetic patients with chronic heart failure.

**Materials and methods:** An observational study was performed. 80 type 2 diabetic patients with chronic heart failure were included. The severity of heart failure was estimated by a 6-minute walk test and by the echocardiography, 48 patients (60%) characterized by III and IV functional class of heart failure. The median of duration of diabetes was 11 years. 40 patients (50%) used oral hypoglycaemic agents, 36 (44%) - insulin as monotherapy or in combination with oral medication, 5 (6%) - on the diet. Follow-up was 6 months. The study protocol included a 3-fold daily Holter electrocardiogram monitoring: at the inclusion visit, at 3 months and 6 months. Patients kept diaries of self-control blood glucose levels and hypoglycaemic events. During the day of Holter monitoring glucose was studied before meals, 2 hours after main meals, at

bedtime and 3 am. Combined simultaneous monitoring (Holter + continuous glucose monitoring) was performed in 20 patients. We used the CGM system by Medtronic MiniMed (USA) and the "Myocard-Holter" (NIMP ESN Ltd, Russia). Ventricular arrhythmias 4-5 and E-D gradations by Myerburg R.J. (2001) were regarded as dangerous. The parameters of glycaemic variability were also examined.

**Results:** 7 patients died during the study. Dangerous ventricular arrhythmias were detected in 42 patients (51 %) in 3-fold Holter monitoring. Glucose less than 5 mmol/l during the day of Holter increased 4.8 times the risk of dangerous arrhythmias ( $p=0.03$ ; logistic regression). Mean Amplitude of Glycaemic Excursion (MAGE) was associated with dangerous ventricular arrhythmias. There is a direct correlation between the level of MAGE and the number of ventricular premature beats ( $r=0.54$ ;  $p=0.02$ , Spearman). MAGE in patients with arrhythmias was 5.3 mmol/l [4.9; 6.3], in cases without arrhythmias - 2.4 mmol/l [2.0; 3.0] ( $p=0.006$ , Mann-Whitney U-test). Prevalence of dangerous ventricular arrhythmias in cases with MAGE > 5 mmol/l was 43 %, in cases with MAGE < 5 mmol/l - only 8 % ( $p=0.02$ , Pearson Chi-square).

**Conclusion:** The high glycaemic variability (MAGE > 5.0 mmol/l) is associated with dangerous ventricular arrhythmias. The high glycaemic variability is a proarrhythmic factor in type 2 diabetic patients with heart failure.

231

### Glycaemic fluctuations and arrhythmias in type 2 diabetes with cardiovascular disease

M. Hanefeld<sup>1</sup>, A. Stahn<sup>1</sup>, E. Henkel<sup>1</sup>, M. Teige<sup>1</sup>, C. Koehler<sup>1</sup>, A. Thomas<sup>2</sup>;  
<sup>1</sup>Center for Clinical Studies, GWT-TUD GmbH, Germany, <sup>2</sup>Medtronic GmbH, Meerbusch, Germany.

**Background and aims:** Patients with type 2 diabetes and cardiovascular disease (CVD) may represent a high risk group for arrhythmias and sudden death in hypoglycemic episodes. Little is known on frequency of asymptomatic hypoglycemia and its relationship to arrhythmias. By parallel measurement of interstitial glucose (i. G.) concentrations with continuous glucose measurement system (CGMS) and long-term ECG recording (LT-ECG) we therefore analysed the following questions in a high risk cohort:

- 1 - Relationship between low i. G. concentration and frequency of arrhythmias
- 2 - Relationship between fluctuation and frequency of arrhythmias
- 3 - Critical arrhythmias and their determinants

**Materials and methods:** 23 patients with type 2 diabetes, HbA<sub>1c</sub> < 9 %, age 50 - 80 years, with proven CVD, treated with insulin and/ or sulfonylurea, were considered. CGMS with Medtronic MiniMed Gold™ system and ECG with Amedtec ECG pro\* were recorded in parallel for 5 days and we analysed the frequency of ventricular extrasystoles and salvos, supraventricular extrasystoles and measured cQT-time. Hypoglycemia by CGMS was defined as modest (3.1-3.9 mmol/L) or severe (<3.1 mmol/L). The patients noticed symptoms of hypoglycemia and arrhythmias in a predefined protocol.

**Results:** The patients recorded only few mild symptomatic hypoglycemia. However we observed a high frequency of silent hypoglycemic episodes by CGMS. No arrhythmias were reported by the patients although we observed serious ventricular arrhythmias like ventricular tachycardias by LT-ECG. Hypoglycemic episodes and arrhythmias were most frequent at night.

**Conclusion:** Patients with CVD on insulin or sulfonylurea treatment represent a high risk for silent hypoglycemia and arrhythmias. Avoidance of excessive glucose fluctuations in glucose lowering treatment might be of importance to protect patients with CVD from arrhythmias.

232

### A short-term improvement of blood glucose control increases aspirin sensitivity in aspirin-resistant type 2 diabetic men

I. Russo, C. Barale, M. Chirio, M. Secchi, M. Viretto, G. Doronzo, L. Mattiello, A. Pagliarino, F. Cavalot, M. Valle, G. Anfossi, M. Trovati;  
 Department of Clinical and Biological Sciences of the Turin University, Internal Medicine and Metabolic Disease Unit, San Luigi Gonzaga Hospital, Orbassano - Turin, Italy.

**Background and aims:** Aspirin is recommended to the majority of patients affected by type 2 diabetes mellitus (T2DM) although the aspirin-induced prevention of cardiovascular events is smaller in diabetes than in the general population. Determinants of the aspirin resistance and of its reversibility in T2DM, however, are poorly clarified. We aimed at verifying whether in T2DM



men a short-term improvement of blood glucose (BG) control influences aspirin resistance.

**Materials and methods:** T2DM men with HbA1c >7.5% (n=37, age: 61.6±1.2 years, known diabetes duration 12.8±1.1 years, BMI: 29.6±0.60 kg/m<sup>2</sup>, HbA1c: 8.9±0.14%), on 100 mg/day aspirin were submitted to a 3-month intervention aiming to reach the best possible BG control by implementation of hypoglycaemic therapy with a careful avoidance of hypoglycemia. No change in hypolipidemic or anti-hypertensive treatment was allowed. Before and after the intervention, platelet sensitivity to aspirin was evaluated by Platelet Function Analyzer-100 (PFA-100): the cut-off for aspirin sensitivity is a closure time >200 sec. Among many other parameters, we evaluated: blood pressure (BP), hemoglobin A1c (HbA1c), fasting and post-prandial BG, lipids, von Willebrand Factor (vWF), a determinant of PFA-100 response. Data are expressed as mean±SEM. To compare subjects within and between the two groups, two factor mix design ANOVA and Spearman's correlation analysis were used.

**Results:** At baseline, aspirin sensitive (n=27, 73%) and aspirin resistant (n=10, 27%) subjects did not significantly differ for age, known diabetes duration, BMI, systolic and diastolic BP, HbA1c, fasting and post-lunch BG, HDL-cholesterol, triglycerides, and vWF, whereas aspirin-resistant subjects presented higher levels of total cholesterol (185.3±8.9 vs 156.6±5.4 mg/dl, p<0.009), LDL-cholesterol (105.3±14.3 vs 70.6±8.7 mg/dl vs, p<0.05) and apo B100 (93.0±4.9 vs 77.2±3.0 mg/dl, p=0.009). BG variables decreased after the intervention in aspirin sensitive and in aspirin resistant patients: i) HbA1c, from 8.8%±0.2% to 7.6%±0.1% (p<0.0001) and from 9.2%±0.3% to 7.6±0.2% (p<0.0001), respectively; ii) fasting BG, from 177.8±7.2 to 153.4±5.6 mg/dl (p<0.002) and from 181.7±11.9 to 134.3±9.1 mg/dl (p<0.0001), respectively; iii) post-lunch BG, from 182.1±9.3 to 144.4±6.9 mg/dl (p<0.0001) and from 186.3±15.3 to 148.0±11.3 mg/dl (p<0.009), respectively. PFA-100 values did not change in patients already aspirin-sensitive at baseline, but significantly improved in the aspirin-resistant-ones, from 149.2±10.4 to 228.5±15.0 sec (p=0.0001). In aspirin-resistant subjects baseline values of PFA negatively correlated with HbA1c (rho=-0.835, p=0.003) and fasting BG (r=-0.806, p=0.05).

**Conclusion:** i) 27% of poorly controlled T2DM men are aspirin resistant; ii) aspirin resistant T2DM men are characterized by higher values of total and LDL-cholesterol and of apoB-100 vs the aspirin sensitive-ones; iii) in aspirin resistant T2DM men, platelet sensitivity to aspirin strongly correlates with the BG control, whose improvement is accompanied by a decrease of aspirin resistance. Thus, BG improvement plays a major role in the reversal of aspirin insensitivity in T2DM men.

*Supported by: EFSD/Sanofi grant to M.T.*

## OP 41 Modifiers and markers for cancer in type 2 diabetes

233

**Short- and long-term survival in Scottish cancer patients with and without co-morbid type 2 diabetes (1997-2007)**

J.J. Walker, The Scottish Diabetes Research Network Epidemiology Group; Centre for Population Health Sciences, The University of Edinburgh, UK.

**Background and aims:** Cancer patients who have pre-existing diabetes at diagnosis are at greater risk of long-term all-cause mortality than those without diabetes. However, relatively few studies have examined how the influence of diabetes on survival varies for specific cancers throughout the time period after cancer is diagnosed. This study investigated short- and long-term survival in Scottish cancer patients with and without Type 2 diabetes (T2DM) for cancer of the breast, lung, prostate and colon/rectum.

**Materials and methods:** Data from a population-based national diabetes register and the Scottish Cancer Registry were used to investigate associations between co-morbid diabetes (at the time of cancer diagnosis) and all-cause survival in the intervals 0 to 1 year, >1 to 2 years, >2 to 5 years and over five years after diagnosis. People in Scotland diagnosed with any of the cancers of interest during 1997 to 2007 were followed up until the end of 2010. Cox regression models incorporating terms representing the interaction between time and presence of T2DM were fitted to estimate the hazard ratio (HR; T2DM vs. no diabetes) in each interval. Separate models were fitted for each combination of sex and cancer site. Estimates were adjusted for age at cancer diagnosis, socio-economic status (SES) and cancer stage at diagnosis (breast and colon/rectum only). All models were re-fitted with additional predictors representing broad categories of cancer treatment (surgery, radiotherapy, chemotherapy). Cumulative incidence functions were calculated and plotted to provide insight into the respective probabilities of death from the index cancer and from any other cause over the period studied.

**Results:** Numbers of patients without diabetes ranged from a minimum of 12,082 (female; cancer of the colon/rectum); the corresponding number with pre-existing T2DM was 948. During the intervals 0 to 1 year and >1 to 2 years post diagnosis, survival did not differ significantly by diabetes status for any cancer. In the interval >2 to 5 years, survival in the T2DM group was poorer in men with prostate cancer (HR 1.28; 95% confidence interval [CI] 1.15 to 1.41) and, marginally, in women with colorectal cancer (HR 1.18; 95% CI 1.01 to 1.38). Over the interval >5 years, significantly elevated risk in the T2DM group was observed for all sex/site combinations except women with lung cancer. Hazard ratios for men in this interval were lowest for prostate cancer (HR 1.33; 95% CI 1.16 to 1.52). For women, the least elevation of risk was for lung cancer (HR 1.31; 95% CI 0.84 to 2.03). Further adjustment for cancer treatment did not materially change the results. Examination of cumulative incidence functions indicates that the respective probabilities of death from the index cancer and from any other cause differ markedly across cancer sites.

**Conclusion:** This study indicates that the presence of co-morbid Type 2 diabetes at the point of cancer detection does not influence survival (in respect of death from any cause) during the first two years after cancer is diagnosed. Over a more extended period, the influence of co-morbid T2DM varies by sex and cancer site, with higher risk generally observed in those with diabetes over the interval beyond 5 years. This elevation of risk may reflect less aggressive cancer therapy received by patients with co-morbid diabetes, and the increased susceptibility of people with diabetes to death from causes other than cancer (e.g. heart disease).

*Supported by: EFSD Diabetes and Cancer grant / SHIP / Wellcome Trust Grant WT086113*

234

**High proinsulin levels are independently associated with 20-year cancer mortality. The Hoorn study**

I. Walraven<sup>1,2</sup>, E. van 't Riet<sup>2</sup>, C.D.A. Stehouwer<sup>3</sup>, B.C. Polak<sup>1</sup>, G. Nijpels<sup>2</sup>, J.M. Dekker<sup>2</sup>;

<sup>1</sup>Ophthalmology, VU Medical Center, Amsterdam, <sup>2</sup>Epidemiology and Biostatistics, VU Medical Center, Amsterdam, <sup>3</sup>Internal Medicine, Maastricht University Medical Center, Netherlands.

**Aims:** High proinsulin levels are associated with all-cause and cardiovascular mortality. It is unclear whether they are associated with cancer mortality

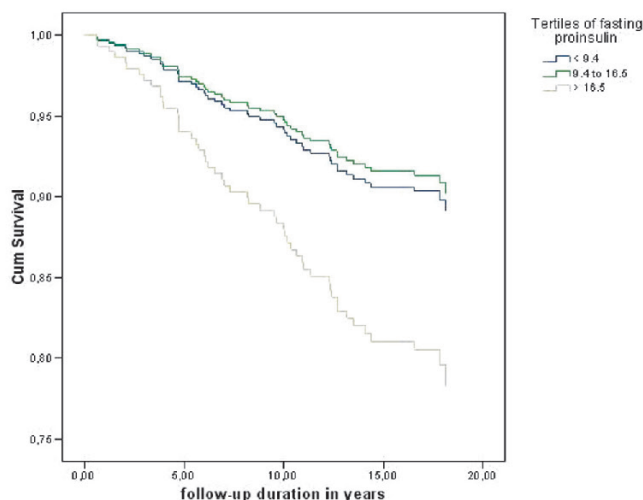
as well. It is shown that proinsulin binds with and activates insulin receptor isoform A, which in turn has been found in cancer cells and mediates the growth effects of pro-insulin-like growth factor II. The aim of this study was to investigate the independent association of proinsulin levels with 20-year cancer mortality in a population-based study.

**Materials and methods:** The current study was performed within the Hoorn Study, a population-based cohort study of glucose tolerance status among Caucasians aged 50–75 years. Fasting proinsulin levels were measured by a double-antibody radioimmunoassay on two separate days. Participants were continuously followed to register mortality, causes of death were derived from medical records. Cox survival analyses were performed to assess the 20-year cancer mortality risk in relation to tertiles of proinsulin levels. All analyses were adjusted for age and sex, with additional adjustments for variables that changed the relationship more than 10%, i. e. glucose tolerance status (WHO 1999), waist circumference, HDL-cholesterol and hypertension. Furthermore, we tested for interaction of glucose tolerance status and sex with proinsulin tertiles.

**Results:** Fasting proinsulin levels were available in 511 participants (baseline mean age 60 years, 41% normal glucose tolerance, 35.7% impaired glucose tolerance and 23.3% diabetes mellitus type 2). Of those, 53 individuals died from cancer. Individuals who died from cancer were older and had significantly higher specific insulin and proinsulin levels at baseline. After adjustment for age and sex, the highest tertile of proinsulin ( $> 16.5$  pmol/L) was significantly associated with cancer mortality [hazard ratio (HR) 1.99 (95% CI 1.04 to 3.82)]. Additional adjustment for possible confounding and/or specific insulin did not substantially change the results [HR 2.16 (95% CI 1.05 to 4.44)] (figure 1). No interaction with glucose tolerance status or sex was observed.

**Conclusion:** In the current study, we showed that fasting proinsulin levels above 16.5 pmol/L as compared to fasting proinsulin levels below 9.4 pmol/L were significantly associated with increased rates of cancer mortality over a 20-year time span. This association was independent of glucose tolerance status, waist circumference, HDL-cholesterol, hypertension and specific insulin. These findings provide population-based evidence for the independent association of high proinsulin levels with cancer mortality.

**Figure 1.** Kaplan-Meier curves of the 20-year cancer mortality risk in relation to tertiles of fasting proinsulin



## 235

### Metformin reduces growth and progression of pancreatic tumours in obese diabetic mice through regulation of cancer stem cell-associated gene expression

V. Cifarelli, S.M. Dunlap, L.M. Lashinger, S.D. Hursting;  
Department of Nutritional Sciences, School of Human Ecology, The University of Texas at Austin, USA.

**Background and aims:** The risk of developing pancreatic cancer (PC) is increased during diabetes mellitus. Epidemiological studies demonstrate that glucose-lowering therapies might impact the risk for PC in type 2 diabetes (T2D) patients. Metformin, an extensively used drug for the treatment of T2D, has been shown to be positively associated with the decreased risk of

PC in T2D patients. However, the mechanism of metformin inhibition of initiation of PC remains unknown. The aim of this study was to investigate the direct effect of metformin on growth and progression of PC using a tumor cell transplant mouse model fed the Diet Induced Obesity (DIO) diet. We analyzed the direct effect of metformin on the pancreatic cancer stem cell (CSC) population and cells that have an epithelial-to-mesenchymal transition (EMT) phenotype. These cells are hypothesized to be the cause of tumor recurrence and metastasis. Since CSCs can expand as sphere-like cellular aggregates, we studied the effect of metformin on the formation of pancreatic tumor sphere formation and associated genetic markers of CSCs and EMT.

**Materials and methods:** Mouse pancreatic cancer cells Panc02 were cultured for 24–72h in presence or absence of metformin (0.5–5mM). CD24+CD44+CD133+ CSCs population was identified by Flow Cytometry. Oct-4 and Notch expression, such as markers required to maintained stem cell identity, was analyzed by qRT-PCR. For the tumor sphere formation assay, Panc02 were seeded in ultra low adherent 6-well plate, using a DMEM/F12 media supplemented with B-27 and N2 growth factors. Tumor spheres were collected by centrifugation and the expression of EMT markers was analyzed by qRT-PCR. C57BL/6 mice (n=50, male) were rendered diabetic by DIO diet (60%kcal from fat). After 15 weeks of diet, metformin (250 mg/kg) was administered in drinking water, whereas control mice received regular water for 4 weeks. Panc02 cells were then injected subcutaneously. Mice continued metformin treatment for the duration of the study and tumor growth was monitored for 4 weeks post-injection.

**Results:** Metformin reduced pancreatic CSCs expression in Panc02 by 25% compared to control after 24h treatment ( $43.6\pm2.9$  vs.  $60.8\pm0.9$  in control;  $p<0.01$ ). The expression of Oct-4 and Notch were also decreased by 60% ( $\pm0.108$ ) and 50% ( $\pm0.002$ ) respectively. EMT regulators, Snail and Zeb-1, were decreased ( $RQ=0.72\pm0.06$  and  $RQ=0.64\pm0.11$ ) after metformin treatment. Metformin inhibited the expression of Vimentin ( $RQ=0.68\pm0.15$ ), TGF- $\beta$  ( $RQ=0.62\pm0.06$ ) and Fibronectin ( $RQ=0.81\pm0.02$ ) (all  $p<0.05$  vs. control) after 24h treatment. Metformin reduced the number ( $11.1\pm3.37$ ;  $p<0.05$ ; vs.  $23.8\pm5.7$  in control) and the size ( $115\mu\text{m}\pm21.9$  vs.  $187\mu\text{m}\pm34.05$  in control;  $p=0.01$ ) of tumor spheres. In diabetic mice, metformin improved hyperglycemia, reduced body weight ( $42.5\text{g}$  control vs.  $40\text{g}$  treated mice) and pancreatic tumor growth, and modulated the expression of EMT markers.

**Conclusion:** Our results indicated that metformin directly affects pancreatic cancer growth and progression by reducing the CSC population and gene expression PC in obese diabetic mice. Metformin reduces pancreatic cancer stem cells expression and affects the growth and progression of pancreatic tumor in diabetic mice.

Supported by: NIH grant R01 CA135386

## 236

### The disturbances of non-specific immunity of type 2 diabetic patients with colon cancer

A. Czech<sup>1</sup>, P. Piatkiewicz<sup>1</sup>, T. Milek<sup>2</sup>, M. Kniotek<sup>3</sup>, M. Bernat-Karpinska<sup>1</sup>, M. Nowaczyk<sup>3</sup>, M. Bernas<sup>1</sup>;

<sup>1</sup>Chair and Department of Internal Diseases and Diabetology, <sup>2</sup>Chair and Department of General and Vascular Surgery, <sup>3</sup>Department of Clinical Immunology, Warsaw Medical University, Poland.

**Background and aims:** Peripheral blood NK cells represent the first line defense in the immune system. Glucose metabolism disorders may influence anticarcinogenic function of these cells. The earlier obtained results revealed significant impairment in activity of NK cells in Type 2 diabetes. The aim of this study was to evaluate the number and cytotoxic activity of NK cells obtained from Type 2 diabetic patients (T2D) with negative family history of cancer, Type 2 diabetic subjects with newly diagnosed untreated colon cancer (T2DCC), and subjects without Type 2 diabetes with newly diagnosed, untreated colon cancer (CC).

**Materials and methods:** Incubation tests were performed in 18 newly diagnosed T2D, naive to any hypoglycaemic drugs, 16 T2DCC cT1-4N0M0 (c-clinical diagnosis based on computer tomography, colonoscopy and histopathology) treated with diet only or oral antidiabetic agents and 16 normoglycemic CC cT1-4N0M0. The control group included 18 metabolically healthy subjects (HS) with negative family history of cancer, matched with age, BMI and waist circumference. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation. The K562 human erythroleukemia cell line was used as the standard target for human NK cytotoxicity assay. K562 were labelled with DIO(3,3-diocetadecyloxycarbocyanine perchlorate). Target and effector cells were added to reach effector/target ratios: 50:1, 12:1. Dead target cells were stained with PI (propidium iodide). After 4 hours of

incubation data was collected for analysis on the Becton-Dickinson FACS-calibur flow cytometer. The data was analyzed using Cell Quest software. The results were compared using Aspin-Welch test.

**Results:** The T2D in comparison to HS had an increased number ( $13,56 \pm 5,9\%$  vs  $9,50 \pm 4,8\%$ ,  $p < 0.05$ ) but decreased activity ( $3,3 \pm 2,5\%$  vs  $9,4 \pm 3,6\%$ ,  $p < 0.01$ ) of NK cells. The CC demonstrated substantially decreased activity ( $2,9 \pm 1,8\%$ ;  $p < 0.01$ ) but similar number ( $8,82 \pm 3,7\%$ ; ns-not statistically significant) of NK cells when compared to the HS. The T2DCC NK cells were characterized by very small cytotoxic activity ( $1,1 \pm 0,7\%$ ;  $p < 0.01$ ) and nearly 2-fold higher number ( $21,24 \pm 7,5\%$ ;  $p < 0,01$ ) when compared to T2D.

**Conclusion:** Type 2 diabetes and colon cancer are associated with disadvantageous alterations of NK cells population leading to the impairment of their cytotoxic activity. The impaired activity of NK cells in Type 2 diabetes can be involved in the increased cancerogenic risk and promote a higher incidence of colon cancer.

*Supported by: Warsaw Medical University*

## OP 42 Changes over time in type 1 diabetes

237

**Long-awaited decrease in the incidence of type 1 diabetes in Finnish children under age 15**

V. Harjutsalo<sup>1</sup>, R. Sund<sup>2</sup>, M. Knip<sup>3</sup>, P.-H. Groop<sup>1</sup>;

<sup>1</sup>Folkhälsan Institute of Genetics, Folkhälsan Research Center, Biomedicum Helsinki, <sup>2</sup>National Institute for Health and Welfare, Service Systems Research Unit, <sup>3</sup>Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland.

**Background and aims:** Increasing incidence in childhood type 1 diabetes has been reported from many countries during the last decades with accelerated increase in some countries. Finland has the highest incidence of type 1 diabetes worldwide, reaching 64.2 per 100 000 people per year in 2005. Our aim was to assess the recent trends in the type 1 diabetes incidence between 2006 and 2010 in Finnish children under the age of 15 years.

**Materials and methods:** Children with newly diagnosed type 1 diabetes in Finland were ascertained from the Hospital Discharge Register, the Drug Reimbursement Register and the Drug Prescription Register during 2006-2010 and compiled by the FinDM diabetes research database. Patients with other types of diabetes were excluded. Age-standardized annual incidence rates and sex and age-specific incidence rates were calculated. Time trends for the incidence rates were estimated by Poisson regression models with sex, age group and year as independent variables. In order to achieve a comprehensive picture of the long-term development of the incidence rate the new data were incorporated into the previous data on incidence (1980-2005) and the trend fluctuation was modeled by Generalized Additive Models.

**Results:** A total of 2 737 children, 1520 boys and 1217 girls, were diagnosed with type 1 diabetes before 15 years of age during 2006-2010. The average age-standardised incidence was 61.2 per 100,000 (95% CI 58.9-63.5), 66.3 (95% CI 63.4-70.1) for boys and 55.7 (95% CI 52.6-59.0) for girls during this period. Incidence decreased each year on average 3.3% (95% CI 0.7-5.8) ( $p = 0.01$ ) from the peak incidence 64.9 per 100 000 per year in 2006 to 56.9 in 2010. The decrease was most striking in the age group 5-9 years. The average age-specific rates were 54.9, 70.4, and 50.0 at ages 0-4 years, 5-9 years, and 10-14-years over the observation period, respectively. Mean age at diagnosis was  $7.03 \pm 3.82$  years in girls and  $7.48 \pm 4.06$  years in boys. Compared to the previous five-year period, between 2001 and 2005, there was no further decrease in age at onset neither in boys nor in girls.

**Conclusion:** The long ongoing increase in the incidence of type 1 diabetes in Finnish children has stopped and the incidence has for the first time decreased since the 1950s. The overall decrease totaled 12.3% from the peak incidence observed in 2006 until the end of 2010. The decrease implies that some environmental exposures that are specific to the early childhood years and that prevailed at the beginning of the 2000s have abated and become less prevalent. However, the incidence is still extremely high in Finnish children and much higher than in any other countries in the world, 56/100 000 per year. Furthermore, the decrease has to be interpreted with caution, since it is still possible that this is only a temporary phenomenon and the incidence may start to increase again.

*Supported by: Folkhälsan Research Foundation and Stockmann Foundation*

238

**Disease progression has changed during the last 30 years among 446 children with newly diagnosed type 1 diabetes located in Scandinavia and Europe**

M.L.M. Andersen<sup>1</sup>, S. Pörksen<sup>1</sup>, P. Hougaard<sup>2</sup>, L.B. Nielsen<sup>1</sup>, J.V. Jørgensen<sup>3</sup>, M. Wallensteen<sup>4</sup>, E. Örtqvist<sup>5</sup>, J.S. Petersen<sup>6</sup>, P. Swift<sup>7</sup>, L. Hansen<sup>1</sup>, H.B. Mortensen<sup>1</sup>;

<sup>1</sup>Pediatrics, Herlev University Hospital, Denmark, <sup>2</sup>Statistics, University of Southern Denmark, Odense, Denmark, <sup>3</sup>Pediatrics, Skejby University Hospital, Århus, Denmark, <sup>4</sup>Pediatrics, Centrallasarettet, Västerås, Sweden, <sup>5</sup>Woman and Child Health, Astrid Lindgrens Children's Hospital, Stockholm, Sweden, <sup>6</sup>Diabetes Biology & Pharmacology, Novo Nordisk A/S, Måløv, Denmark, <sup>7</sup>Children's Hospital, Leicester Royal Infirmary, UK.

**Background and aims:** The purpose of the study was to assess whether the rate of decline in stimulated C-peptide (SCP) from 3-12 months after diag-



nosis in 4 independent paediatric cohorts with different geographic locations has changed over an interval of 30 years. Furthermore the study aimed to examine covariates that predict disease progression rates

**Material and methods:** The rate of decline in SCP levels the first 12 months after diagnosis was compared in 4 paediatric cohorts with type 1 diabetes: 39 children (9.0 yr (range 1.0–17.0 yr), 1982–1985) from the Wallensteen Swedish cohort; 29 placebo treated children (11.6 yr (range 7.4–15.5 yr); 1995 to 1998) from the Örtqvist Swedish Cohort; 251 children (9.0 yr (range 0.2–16.3 yr), 1999 to 2000) from the Hvidoere Remission Phase Cohort and 127 children (10.1 yrs (range 0.6–16.6 yr), 2004 to 2005) from the Danish Remission Phase Cohort. Patients were followed 12 months after diagnosis with a 90-minutes liquid mixed meal challenge after 1, 3, 6, 9, 12 months (if available). Random coefficient regression models were used for analysing the relationship between SCP decline and influencing factors such as age and BMI. At present we perform statistical analyzes including 78 placebo treated children with new onset type 1 diabetes from the placebo groups in the TrialNet cohorts collected in the period 2004–2009

**Results:** Maximum values of SCP were reached 3 months after diagnosis and then declined gradually. The latest cohort had significantly higher SCP values initially ( $p \leq 0.0001$ ) and significantly faster rate of decline in SCP ( $p = 0.05$ ) than the other 3 cohorts, when adjusted for age. The mean rate of decline in SCP for a 10 years old child was 7.7% (Wallensteen), 6.3% (Örtqvist), 7.8% (Hvidoere) and 10.7% (Danish) for the four cohorts, respectively. There was a linear effect of age on the rate of decline in SCP, suggesting 0.7 %/month/year (0.4–0.9) less decline per year of age of the child ( $p < 0.0001$ ). BMI at 3 months had no significant effect on the rate of decline in SCP ( $p = 0.36$ ), by contrast BMI is positively associated to the level of SCP suggesting 7.3 % (3.7–10.8 %) higher C-peptide per 1 kg/m<sup>2</sup> increase in BMI ( $p = 0.0002$ ). The proportion of patients in partial remission as assessed by insulin dose adjusted HbA<sub>1c</sub> (IDAA1c)  $\leq 9$  differed from 35.3% to 47.8% after 6 months and declined to 17.8 to 21.6% 12 months after diagnosis with no significant difference between the cohorts.

**Conclusion:** T1D disease progression seems to have changed during the last 30 years with a tendency toward a more rapid rate of decline in SCP the first 12 months after diagnosis in the latest Danish cohort from 2005. Thus, the environmental factors that possibly explain the increased incidence in T1D in this period might also influence the progression rate of the disease. The rate of decline in SCP is dependent of age but not BMI. Despite higher levels of SCP in the Danish cohort, there are no significant differences in the proportion of children in partial remission.

## 239

### Current mortality risks in the adult type 1 diabetes population of Scotland: high risks at young ages

H.M. Colhoun, on behalf of the Scottish Diabetes Research Network Epidemiology Group; University of Dundee, UK.

**Background and aims:** In a dataset encompassing the entire population of Scotland we examined the current relative risks for mortality and causes of death in people with type 1 diabetes compared with the non-diabetic population.

**Materials and methods:** The Scottish Care Information - Diabetes Collaboration Database that is used in clinical care across Scotland, provided data for 21,789 people with type 1 diabetes aged  $\geq 20$  years. The National Records of Scotland provided the annual numbers of deaths from 2005–2007 for the non-diabetic population of Scotland aged  $\geq 20$  years (3.75 million) and provided numbers and cause of death for those with type 1 diabetes (1,030 deaths). The age adjusted mortality incidence rate ratio (IRR) associated with type 1 diabetes was estimated using Poisson regression.

**Results:** During 2005–2007 the mortality IRR (95% CI) for those with, relative to those without, type 1 diabetes was 2.58 (95% Confidence interval (CI); 2.23–2.98,  $p < 0.001$ ) in men and 2.71 (95% CI 2.18–3.38,  $p < 0.001$ ) in women. The IRR decreased with age being 5.43 (95%CI: 3.50, 8.42) overall at age 20–29 years and 1.82 (95%CI:1.61, 2.06) age  $\geq 70$  years. Of the 123 deaths in 10,173 people  $< 40$  years with type 1 diabetes (rate 4.8/1000 person years at risk), the top three underlying causes were diabetes mellitus (41.4% : of which coma or ketoacidosis accounted for 34 of 51 deaths), other metabolic disorders (12.2% : 15 deaths) and circulatory disease (11.4%: 14 deaths). Among the 907 deaths in those with T1DM age  $\geq 40$  the leading causes were circulatory disease (38.5%: 349 deaths), diabetes mellitus (20.6%: of which coma and ketoacidosis accounted for 37 and renal complications 47 of 187 deaths) and

neoplasm (17.0%: 154 deaths). Overall just 63% of death certificates in those  $< 40$  years and 69% in those  $\geq 40$  years mentioned diabetes.

**Conclusion:** Despite advances in care, type 1 diabetes continues to be associated with substantial elevations in total mortality rates in both sexes with very high relative risks at young ages.

Supported by: Wellcome Trust via Scottish Health Informatics Programme Grant (WT086113)

## 240

### HbA<sub>1c</sub> and OGTT plasma glucose levels start to rise during the last year before diagnosis in children with multiple autoantibodies and increased genetic risk for type 1 diabetes

O. Helminen<sup>1</sup>, M.-R. Hautakangas<sup>1</sup>, N. Haatanen<sup>1</sup>, T. Pokka<sup>1</sup>, J. Ilonen<sup>2</sup>, O. Simell<sup>3</sup>, M. Knip<sup>4</sup>, R. Veijola<sup>1</sup>;

<sup>1</sup>Dept of Pediatrics, University of Oulu, <sup>2</sup>Immunogenetics Laboratory, University of Turku, <sup>3</sup>Dept of Pediatrics, University of Turku, <sup>4</sup>Inst of Clin Medicine, University of Helsinki, Finland.

**Background and aims:** Few data is available on the development of glucose intolerance before diagnosis of type 1 diabetes. We pictured HbA<sub>1c</sub> and OGTT values over five years before diagnosis in children with advanced beta cell autoimmunity.

**Materials and methods:** Children with increased HLA-conferred risk of type 1 diabetes (T1D) have participated in the type 1 Diabetes Prediction and Prevention (DIPP) Study in Finland. During follow-up the subjects have been screened for islet cell (ICA), glutamic acid decarboxylase (GADA), insulinoma associated antigen-2 (IA-2A) and insulin autoantibodies (IAA) at ages of 3, 6, 12, 18 and 24 months and annually thereafter. Children seroconverting positive for any of the autoantibodies were transferred to a schedule with 3 month (0.25 yr) intervals including also HbA<sub>1c</sub> measurements. When multiple positive autoantibodies occurred oral glucose tolerance tests (OGTT) were performed annually. Our study population consists of 100 children participating in the DIPP follow-up at Oulu University Hospital and who have progressed to T1D before Jan 1, 2012. HbA<sub>1c</sub> study population includes 65 children with HbA<sub>1c</sub> available at diagnosis and at least once during the previous 12 months. OGTT study population consists of 65 children who had been tested with OGTT at least once before diagnosis.

**Results:** Seroconversion to at least one autoantibody occurred at mean age of 2.30 yrs (SD 1.68) and multipositivity at 2.69 yrs (SD 1.88). HbA<sub>1c</sub> and OGTT values before diagnosis are shown in Fig. 1. HbA<sub>1c</sub> values begun to rise 0.75–0.99 yrs before diagnosis when the mean HbA<sub>1c</sub> was 5.66 % (SD 0.49) compared to the mean of 5.39% (SD 0.35) measured between 1.00–4.99 yrs before diagnosis ( $p < 0.001$  in paired sample t-test). Rise in HbA<sub>1c</sub> continued towards diagnosis (mean HbA<sub>1c</sub> 5.74%, SD 0.45 during 0.50–0.74 yrs prior to diagnosis,  $p = 0.04$  in comparison with 5.66% during the previous 0.25 yrs period). OGTT 0 hour and 2 hour glucose levels also rose before diagnosis. Both 0 hour and 2 hour mean values showed significant rise between periods 1.00–4.99 yrs (0 hour: 4.15 mmol/l, SD 0.47; 2 hour: 5.60 mmol/l, SD 1.36) and 0.50–0.99 yrs prior to diagnosis (0 hour: 4.54 mmol/l, SD 0.82; 2 hour: 6.90 mmol/l, SD 2.25);  $p = 0.006$  (0 hours) and  $p = 0.014$  (2 hours). A steep rise continued in 2 hour values when coming closer to diagnosis (mean 2 hour glucose 8.75 mmol/l, SD 4.07 during 0.05–0.49 yrs before diagnosis;  $p = 0.024$  compared to 6.90 mmol/l during the previous 0.50–0.99 yrs period), but the mean 0 hour glucose levels were not significantly different (4.52 mmol/l, SD 0.85 vs. 4.54 mmol/l, SD 0.85, respectively;  $p = 0.105$ ).

**Conclusions:** HbA<sub>1c</sub> and plasma glucose levels in OGTT start to rise as early as 1 year before diagnosis the increase becoming steeper towards the onset of T1D.

## OP 43 SGLT-2 inhibitors

### 241

#### Systematic review and meta-analysis of the efficacy and safety of SGLT2 inhibitors in patients with type 2 diabetes mellitus

A. Tsapas<sup>1,2</sup>, D. Vassilakou<sup>1</sup>, E. Athanasiadou<sup>1</sup>, T. Karagiannis<sup>1</sup>, D.R. Matthews<sup>2,3</sup>,

<sup>1</sup>2nd Medical Department, Aristotle University Thessaloniki, Greece, <sup>2</sup>Harris Manchester College, University of Oxford, UK, <sup>3</sup>Oxford Centre for Diabetes, Endocrinology and Metabolism, UK.

**Background and aims:** Sodium-glucose co-transporter 2 (SGLT2) inhibitors are a novel class of oral anti-hyperglycaemic drugs that reduce renal glucose reabsorption. Their therapeutic role in type 2 diabetes has been tested in clinical trials. We performed a systematic review and meta-analyses to assess their efficacy and safety versus placebo or other antidiabetic medications.

**Materials and methods:** We searched Medline, Embase and the Cochrane Library, without any language restrictions, for randomised trials of more than 12 weeks duration that compared an SGLT2 inhibitor with placebo or any other hypoglycaemic medication for type 2 diabetes mellitus. Additionally, we searched websites of pharmaceutical companies, public registries of clinical trials and abstracts of major scientific meetings. Our primary outcome was glycaemic efficacy assessed by the change from baseline in HbA<sub>1c</sub>. Secondary efficacy outcomes included changes in body weight and systolic blood pressure, and percentage of patients achieving HbA<sub>1c</sub> <7%. Safety outcomes included incidence of any hypoglycaemia, adverse events of special interest (urinary and genital tract infections), discontinuation rate and overall cardiovascular morbidity. We conducted meta-analyses using an inverse variance random effects model. Heterogeneity was assessed by the I<sup>2</sup> statistic. Risk of bias was assessed with the Cochrane risk of bias tool.

**Results:** Our search identified 21 eligible trials. Sixteen studies (duration 12–104 weeks, 5030 patients) provided adequate data and were used in our meta-analyses. SGLT2 inhibitors were associated with a greater decline in HbA<sub>1c</sub> compared with placebo (Table). Risk for hypoglycaemia was similar between SGLT2 inhibitors and placebo (risk ratio [RR] 1.10, 95% confidence interval [CI] 0.92 to 1.30) but smaller compared to active comparators (RR 0.24, 95% CI 0.06 to 0.98). No difference in the incidence of urinary tract infections was evident between SGLT2 inhibitors and placebo (RR 1.26, 95% CI 0.94 to 1.70). However, SGLT2 inhibitors were associated with an increased risk for urinary tract infections compared with active comparators (RR 1.51, 95% CI 1.08 to 2.09), and for genital tract infections compared both with placebo (RR 3.28, 95% CI 2.19 to 4.90) or other hypoglycaemic medications (RR 4.57, 95% CI 2.80 to 7.45).

**Conclusion:** Existing data support that SGLT2 inhibitors have a favourable effect on HbA<sub>1c</sub>, weight, systolic blood pressure and incidence of hypoglycaemia. However, they are associated with a significantly increased risk for urinary and genital tract infections.

Type of comparison	Change in HbA <sub>1c</sub> (%) from baseline		Change in body weight (kg) from baseline		Change in SBP (mm Hg) from baseline	
	No of studies/ participants analysed	WMD [95 % CI]; I <sup>2</sup>	No of studies/ participants analysed	WMD [95 % CI]; I <sup>2</sup>	No of studies/ participants analysed	WMD [95 % CI]; I <sup>2</sup>
SGLT2 inhibitors vs Placebo, first line treatment	4 / 532	-0.77 [-0.96, -0.57]; 61%	4 / 532	-1.45 [-2.42, -0.47]; 81%	3 / 396	-6.67 [-9.84, -3.49]; 41%
SGLT2 inhibitors vs Placebo, add on treatment	10 / 2338	-0.59 [-0.66, -0.51]; 0%	10 / 2440	-1.92 [-2.18, -1.66]; 0%	7 / 1752	-2.96 [-4.33, -1.59]; 16%
SGLT2 inhibitors vs other hypoglycaemic medication (as first line or add on treatment)	6 / 1792	-0.07 [-0.21, 0.07]; 46%	6 / 1838	-2.03 [-3.51, -0.54]; 95%	5 / 1536	-3.32 [-4.59, -2.04]; 0%

SGLT2 = sodium glucose transporter 2; HbA<sub>1c</sub> = glycated haemoglobin; SBP = systolic blood pressure; WMD = weighted mean difference; CI = confidence interval

### 242

#### Dapagliflozin does not impact renal function in patients with type 2 diabetes

A. Ptaszynska<sup>1</sup>, A.-G. Chalamandaris<sup>2</sup>, J. Sugg<sup>3</sup>, K. Johnsson<sup>3</sup>, S. Parikh<sup>3</sup>, J.F. List<sup>1</sup>,

<sup>1</sup>Bristol-Myers Squibb, Princeton, USA, <sup>2</sup>Bristol-Myers Squibb, Braine-l'Alleud, Belgium, <sup>3</sup>AstraZeneca, Wilmington, USA.

**Background and aims:** Dapagliflozin (DAPA), a sodium-glucose cotransporter 2 inhibitor (SGLT2) in development for patients with type 2 diabetes (T2DM), decreases hyperglycemia by inhibiting renal glucose reabsorption, reduces weight, and acts as a mild diuretic with modest blood pressure reduction. Due to its renal mechanism, DAPA's effect on renal function was thoroughly evaluated.

**Materials and methods:** Data were collected from 12 placebo (PBO)-controlled randomized studies involving T2DM patients receiving PBO or DAPA 2.5, 5, or 10 mg. Adverse events (AEs) and lab tests were analyzed up to 24 wk (>4000 patients) for all 12 studies and beyond 24 wk (>3000 patients) for 6 of these 12 studies. Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease equation.

**Results:** In the 12 placebo-controlled studies, approximately one-third (36.9–39.7%) of patients had baseline (BL) eGFR > 90 mL/min/1.73m<sup>2</sup>, half (52.8–54.4%) had BL mean eGFR ranging from ≥60 to <90 mL/min/1.73m<sup>2</sup> and 7.5–9.3% of the patients had BL eGFR ranging from ≥30 to <60 mL/min/1.73m<sup>2</sup>. At week 1, eGFR decreased from BL by -2.75, -2.92, and -4.15 mL/min/1.73m<sup>2</sup> for dapagliflozin 2.5, 5, and 10 mg, respectively, but returned to or above BL by Wk 24. In the 6 studies, a similar pattern was observed up to Wk 24 and was maintained to Wk 102. Mean serum creatinine (Cr) changed minimally (±0.01 and ±0.022 mg/dL) from BL to Wk 24 and Wk 102, respectively, in all groups. DAPA had no adverse effect on albuminuria, assessed as urinary albumin: Cr ratio or shift in normal, micro-, or macro- albuminuria category through 102 wk. Renal AEs in the DAPA group were similar to PBO through 24 wk but were reported slightly more frequently through 102 wk (Table). The most commonly reported renal AE was serum creatinine increase; there were no reports of acute nephrotoxicity through 102 wk. The moderate renal impairment (BL eGFR ≥30 and <60 mL/min/1.73m<sup>2</sup>) subgroup (n=74–107) had the highest proportion of patients with renal adverse events compared to the subgroups with BL eGFR ≥60 to <90 and ≥90 mL/min/1.73m<sup>2</sup> up to 24 wk. Few AEs corresponding to volume depletion were reported up to 24 wk (Table). At Wk 24, mean decreases in seated blood pressure (both systolic and diastolic) with DAPA were larger compared to PBO, with no apparent dose dependence, and persisted up to 102 wk. No mean changes from BL in serum electrolytes (Na, K, HCO<sub>3</sub>, or Cl) were observed with DAPA and PBO in either analysis period; slight increases were seen in serum Mg and phosphorus at Wk 24 but not Wk 102.

**Conclusion:** DAPA treatment is not associated with increased risk of acute renal toxicity or deterioration of renal function. In addition to blood pressure lowering associated with DAPA, improved glycemic control and effects of weight reduction may support preservation of kidney function in patients with T2DM.

Renal Related Adverse Events (AEs): Data are n (%) including data after rescue								
	Up to 24 weeks				Up to 102 weeks			
	PBO	DAPA 2.5mg	DAPA 5mg	DAPA 10mg	PBO	DAPA 2.5mg	DAPA 5mg	DAPA 10mg
	N=1393	N=814	N=1145	N=1193	N=785	N=625	N=767	N=859
Serious AEs of renal impairment	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.3)	1 (0.2)	1 (0.1)	0 (0)
AEs of renal impairment leading to discontinuation	3 (0.2)	2 (0.2)	3 (0.3)	5 (0.4)	3 (0.4)	5 (0.8)	7 (0.9)	8 (0.9)
AEs related to renal function	12 (0.9)	11 (1.4)	15 (1.3)	11 (0.9)	13 (1.7)	15 (2.4)	14 (1.8)	16 (1.9)
-Blood creatinine increase	7 (0.5)	6 (0.7)	6 (0.5)	9 (0.8)	5 (0.6)	8 (1.3)	6 (0.8)	9 (1.0)
-GFR decrease	0 (0)	1 (0.1)	3 (0.3)	1 (0.1)	1 (0.1)	1 (0.2)	1 (0.1)	1 (0.1)
Hypotension/Dehydration/Hypovolemia	5 (0.4)	10 (1.2)	7 (0.6)	9 (0.8)	6 (0.8)	10 (1.6)	8 (1.0)	13 (1.5)

Clinical Trial Registration Number: NCT00263276, NCT00972244, NCT00528372, NCT00736879, NCT00528879, NCT00855166, NCT00357370, NCT00680745, NCT00683878, NCT00673231, NCT00643851, NCT00859898  
Supported by: BMS and AZ

## 243

# Efficacy and safety of canagliflozin, a sodium glucose co-transporter 2 inhibitor, compared with sitagliptin in patients with type 2 diabetes on metformin plus sulphonylurea

G. Scherthaner<sup>1</sup>, J. Gross<sup>2</sup>, M. Fu<sup>3</sup>, J. Yee<sup>3</sup>, M. Kawaguchi<sup>3</sup>, W. Canovatchel<sup>3</sup>, G. Meininger<sup>3</sup>

<sup>1</sup>Rudolfstiftung Hospital-Vienna, Austria, <sup>2</sup>Hospital de Clinicas de Porto Alegre, Brazil, <sup>3</sup>Janssen Research & Development, L.L.C., Raritan, USA.

**Background and aims:** Canagliflozin (CANA) is an inhibitor of the sodium glucose co-transporter 2 being developed for the treatment of patients with type 2 diabetes mellitus (T2DM). This 52-week study evaluated the efficacy and safety of CANA 300 mg compared with sitagliptin (SITA) in subjects with T2DM inadequately controlled with metformin (MET) plus a sulphonylurea (SU).

**Materials and methods:** In this randomised, double-blind, active-controlled, Phase 3 study (N = 755), subjects with T2DM inadequately controlled with MET + SU received CANA 300 mg or SITA 100 mg once daily. Changes from baseline in glycaemic and other efficacy endpoints were assessed at Week 52. Adverse events (AEs) were recorded throughout the study.

**Results:** Mean baseline characteristics were similar across groups (age, 56.7 years; HbA<sub>1c</sub>, 8.1%; body weight, 88.3 kg; body mass index, 31.6 kg/m<sup>2</sup>). As shown in the Table, HbA<sub>1c</sub> was reduced from baseline at 52 weeks with CANA 300 mg and SITA 100 mg. CANA 300 mg demonstrated non-inferiority as well as superiority to SITA 100 mg in reducing HbA<sub>1c</sub> ( $P < 0.001$ ). CANA 300 mg provided consistently lower HbA<sub>1c</sub> values over 52 weeks while HbA<sub>1c</sub> increased with SITA 100 mg after Week 12. CANA 300 mg also showed greater reduction in body weight and improvement in fasting plasma glucose (FPG) and systolic blood pressure (BP) compared with SITA 100 mg. CANA 300 mg showed numerical improvement in high-density lipoprotein cholesterol (HDL-C) and was associated with a slight increase in low-density lipoprotein cholesterol (LDL-C) compared with SITA 100 mg.

Table. Summary of Efficacy Endpoints at Week 52 (LOCF)

Parameter <sup>a,b</sup>	CANA 300 mg (N = 377)	SITA 100 mg (N = 378)
HbA <sub>1c</sub> change, %	-1.03 (0.05)	-0.66 (0.05)
Difference vs SITA	-0.37 (-0.50, -0.25) <sup>c,d</sup>	
FPG change, mmol/L	-1.7 (0.1)	-0.3 (0.1)
Difference vs SITA	-1.3 (-1.7, -1.0) <sup>d,e</sup>	
Body weight % change	-2.5 (0.2)	0.3 (0.2)
Difference vs SITA	-2.8 (-3.3, -2.2) <sup>d,e</sup>	
Systolic BP change, mmHg	-5.1 (0.7)	0.9 (0.7)
Difference vs SITA	-5.9 (-7.6, -4.2) <sup>d,e</sup>	
Diastolic BP change, mmHg	-3.0 (0.4)	-0.3 (0.4)
Difference vs SITA	-2.7 (-3.8, -1.7)	
Triglycerides % change	9.6 (2.8)	11.9 (2.9)
Difference vs SITA	-2.3 (-9.8, 5.3) <sup>f</sup>	
LDL-C % change	11.7 (1.8)	5.2 (1.8)
Difference vs SITA	6.4 (1.7, 11.2)	
HDL-C % change	7.6 (0.9)	0.6 (0.9)
Difference vs SITA	7.0 (4.6, 9.3) <sup>g</sup>	
LDL-C/HDL-C % change	6.1 (2.0)	7.2 (2.0)
Difference vs SITA	-1.1 (-6.3, 4.2)	

LOCF, last observation carried forward; SE, standard error; CI, confidence interval; ANCOVA, analysis of covariance; NS, not significant. <sup>a</sup>Least squares mean (SE) change from baseline using ANCOVA and SITA-subtracted mean (95% CI) values; <sup>b</sup>P values are reported for pre-specified comparisons only; <sup>c</sup>Upper limit of 95% CI less than pre-specified non-inferiority margin of 0.3% for the comparison to SITA; <sup>d</sup>Upper limit of 95% CI <0.0% for the comparison to SITA; <sup>e</sup>P <0.001 vs SITA; <sup>f</sup>P = NS vs SITA; <sup>g</sup>Not significant due to multiplicity control.

The overall incidence of AEs was similar with CANA 300 mg (76.7%) and SITA 100 mg (77.5%). Rates of serious AEs (6.4% vs 5.6%) and AE-related discontinuations (5.3% vs 2.9%) were low and similar for CANA 300 mg and SITA 100 mg. Incidences of AEs consistent with superficial genital fungal infections were higher with CANA 300 mg than with SITA 100 mg in women (15.3% vs 4.3%) and men (9.2% vs 0.5%). No differences were observed between CANA 300 mg and SITA 100 mg in the incidences of urinary tract infections (4.0% vs 5.0%) and osmotic diuresis-related AEs (eg, pollakiuria; <3% per specific AE). The proportion of subjects with ≥1 hypoglycaemia episode were similar with CANA 300 mg (43.2%) and SITA 100 mg (40.7%).

**Conclusion:** CANA 300 mg showed improvements in glycaemic control, body weight reduction, and systolic BP over 52 weeks compared with SITA 100 mg, and was well tolerated in subjects with T2DM inadequately controlled with MET + SU.

Clinical Trial Registration Number: NCT01137812

Supported by: Janssen Research & Development, L.L.C.

## 244

# Canagliflozin lowers postprandial plasma glucose and insulin excursions by delaying intestinal glucose absorption in addition to increasing urinary glucose excretion

S. Mudaliar<sup>1</sup>, T.P. Ciaraldi<sup>1</sup>, S. Sha<sup>2</sup>, A. Ghosh<sup>2</sup>, D. Polidori<sup>2</sup>, R.R. Henry<sup>1</sup>

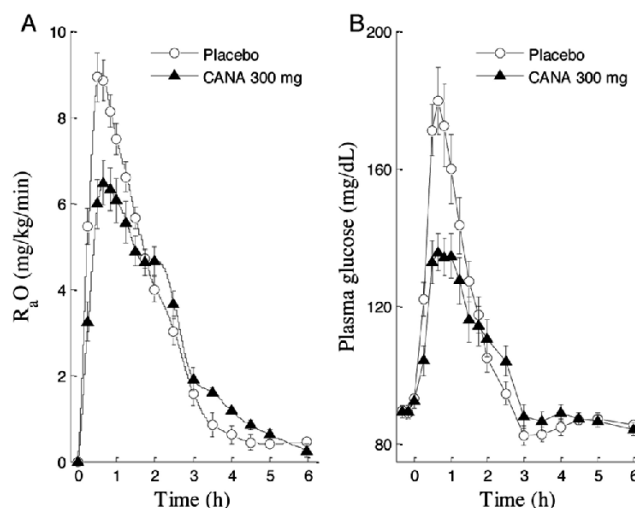
<sup>1</sup>University of California, San Diego, <sup>2</sup>Janssen Research & Development, L.L.C., Raritan, USA.

**Background and aims:** Canagliflozin (CANA), a potent sodium glucose co-transporter 2 (SGLT2) inhibitor, is also a low-potency SGLT1 inhibitor. During drug absorption, intestinal CANA levels post-dose may be sufficiently high to transiently inhibit intestinal SGLT1. This study tested the hypothesis that CANA 300 mg would delay intestinal glucose absorption in healthy subjects during a mixed-meal tolerance test (MMTT).

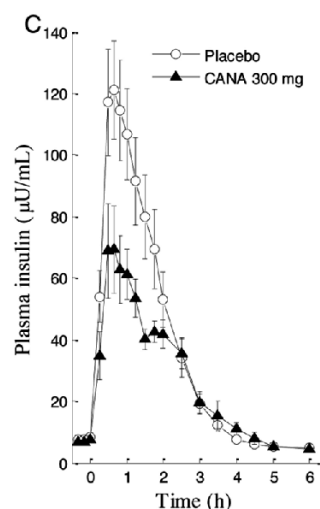
**Materials and methods:** A 2-period crossover study in 20 healthy subjects (mean ± standard deviation [SD] age = 26 ± 6 yr; body weight = 78 ± 10 kg) assessed the effects of a single 300-mg dose of CANA on intestinal glucose (G) absorption using a dual tracer method (IV <sup>3</sup>H-G and oral <sup>14</sup>C-G). Placebo (PBO) or CANA was given 20 min prior to a 600 kcal MMTT containing 75 g of G and 960 mg of acetaminophen. Plasma G, <sup>3</sup>H-G, <sup>14</sup>C-G, acetaminophen, and insulin were measured frequently for 6 h. These values were used to calculate the rate of appearance in plasma of oral G (R<sub>a</sub>G), endogenous G production, and G disposal; acetaminophen concentrations were used as an index of gastric emptying. Plasma glucose-dependent insulinotropic peptide (GIP), peptide YY (PYY), and glucagon like peptide-1 (GLP-1) were measured over the first 2 h and incremental areas under the curves (AUCs) were calculated.

**Results:** CANA was well tolerated. CANA delayed R<sub>a</sub>G (Figure A) and reduced cumulative R<sub>a</sub>G (AUC R<sub>a</sub>G) by 31% at 1 h (geometric mean PBO = 381 vs CANA = 264 mg/kg;  $P < 0.001$ ) and by 20% at 2 h (PBO = 723 vs CANA = 576 mg/kg;  $P = 0.002$ ); this was nearly matched by increased R<sub>a</sub>G over 2–6 h, so that AUC R<sub>a</sub>G over 0–6 h was only approximately 6% lower for CANA than PBO (PBO = 1018 vs CANA = 960 mg/kg;  $P = 0.003$ ), consistent with the lack of notable malabsorption. Plasma acetaminophen concentrations were reduced by approximately 10% over 0–2 h in CANA-treated subjects, suggesting a modest delay in gastric emptying. CANA also increased urinary glucose excretion (UGE) over 0–2 h (PBO <0.2 g; CANA = 6 ± 3 g;  $P < 0.001$ ) and 2–6 h (PBO <0.1 g; CANA = 12 ± 4 g;  $P < 0.001$ ). Total G disposal over 0–6 h was similar for PBO and CANA (PBO = 130 g vs CANA = 132 g;  $P = 0.78$ ). Treatment with CANA decreased plasma GIP (PBO = 63.5 vs CANA = 30.1 pM-h;  $P < 0.001$ ) and increased plasma PYY (PBO = 13.6 vs CANA = 21.9 pM-h;  $P = 0.01$ ) and total GLP-1 (PBO = 10.1 vs CANA = 13.7 pM-h;  $P = 0.07$ ).

**Conclusion:** CANA reduces postprandial plasma G and insulin excursions (Figures B and C) by both increasing UGE (due to renal SGLT2 inhibition) and delaying R<sub>a</sub>G (likely due to intestinal SGLT1 inhibition, with a possible additional contribution from delayed gastric emptying). The changes observed in the gut peptides in CANA-treated subjects (reduced GIP and increased PYY and GLP-1) suggest reduced G absorption from the upper intestine and increased absorption from the lower intestine, consistent with the hypothesis that CANA treatment transiently inhibits intestinal SGLT1.







Clinical Trial Registration Number: NCT01173549  
Supported by: Janssen Research & Development, L.L.C.

## OP 44 Mechanisms of incretin action

### 245

#### Mechanisms of glucose lowering in type 2 diabetes patients treated with sitagliptin alone or in combination with metformin: a double tracer study

C. Solis-Herrera, C. Triplitt, J. Garduno-Garcia, J. Adams, R.A. DeFronzo, E. Cersosimo;  
Medicine/Diabetes, University of Texas, San Antonio, USA.

**Background and aims:** Little is known about glucose lowering mechanisms of sitagliptin and metformin in type 2 diabetes treatment. We examined glucose kinetics and hormonal changes in response to sitagliptin (S), metformin (M), used either as monotherapy or in combination (S+M).

**Materials and methods:** Sixteen T2DM (age~52yrs, 10F/6M, 1yr duration, BMI~32 kg/m<sup>2</sup>, HbA<sub>1c</sub>=8.4±1.2%) were randomized to 4 therapy periods (6wks + 2wk washout) with either Placebo (P), (M), (S) or (S+M) at maximum doses. After each 6wk period, they received a meal tolerance test (MTT, 600 kcal, 20g protein, 25g fat and 75g glucose) labeled with [<sup>14</sup>C-glu] to calculate oral glucose appearance rates (RaO); [<sup>3</sup>H-glu] IV infusion for glucose appearance rates (RaT) & disappearance (RdT). Endogenous glucose production (EGP) equals RaT-RaO. Fasting plasma glucose (FPG), insulin (FPI), glucagon (FPGn) and post-MTT levels were measured over 360 minutes. Plasma incretin levels (intact GLP-1 and GIP) and insulin secretory rate (ISR) (plasma C-peptide deconvolution) were also determined.

**Results:** FPG decreased from 165±10 (P) to 145±6 (M), to 149±9 (S), and to 125±5 mg/dl (S+M); mean post-MTT glucose levels in (P) of 208±15 mg/dl decreased to 181±11 (M), 182±13 (S), and 155±9 (S+M) [p<0.01]. Basal HGP fell from 2.1±0.1 (P) to 1.9±0.2 (M), 1.8±0.1 (S), and to 1.5±0.1 mg/kg.min (S+M) [p<0.05]. Mean RaT post-MTT was lower in (S)=2.5±0.1, (M)=2.7±0.1 and (S+M)=2.3±0.1 than in (P)=2.9±0.2 mg/kg.min [p<0.05]. RaO was similar after all therapies (~1.9-2.3 mg/kg.min), therefore the decline in RaT was due to a drop in HGP post-MTT from 0.8±0.1 (P) to 0.4±0.1 (M), 0.6±0.1 (S) and 0.4±0.1 (S+M) [p<0.05]. Mean RdT post-MMT was equal (~2.1-2.3 mg/kg.min). FPI did not change (~12 µU/ml) in any group but the peak ISR (~13 pmol/kg.min) during MTT was higher in (S+M) than (M)=11±1, (S)=11±1 and (P)=10±1, reflecting a greater rise in mean post-MTT insulin in (S+M)=76±9 vs. (P)=51±9, (S)=52±8 and (M)=56±3 µU/ml, [p<0.05]. FPGn did not change (~70 pg/ml) in any group but greater suppression in plasma glucagon post-MTT was seen in (S+M)=32±3% and (S)=26±3% vs. (P)=14±2% and (M)=13±4%, [p<0.05]. Fasting intact GLP-1 (pM) was higher in S (10.1±1.4) and S+M (16.9±2.2) than P (6.9±1.0) and M (5.5±0.6), and mean post-MMT levels also were higher in S (24.6±1.8) and S+M (32.6±1.6) than P (11.7±0.8) and M (13.7±1.0), [p<0.001]. Fasting plasma GIP (~38-49 pg/ml) and the 4-fold post-MMT increase (~153-163 pg/ml) were equal in all groups.

**Conclusion:** Our findings indicate that sitagliptin and metformin reduce fasting and post-meal hyperglycemia to the same extent, via different mechanisms. Metformin directly suppresses hepatic glucose production whereas sitagliptin exerts a comparable effect indirectly by increasing plasma GLP-1 with significant glucagon inhibition. In addition to these mechanisms both agents (S+M) exert greater glucose lowering effect by also stimulating post-meal insulin secretion.

Supported by: Merck Co & UTHSCSA-UHS/TDI

### 246

#### Degree of DPP-4 inhibition after sitagliptin treatment estimated from intact and total GLP-1 and GIP responses to oral glucose in patients with type 2 diabetes

I. Vardarli<sup>1</sup>, E. Arndt<sup>1</sup>, C.F. Deacon<sup>2</sup>, J.J. Holst<sup>2</sup>, L.D. Köthe<sup>1</sup>, O. Baranov<sup>1</sup>, M.A. Nauck<sup>1</sup>;

<sup>1</sup>Diabeteszentrum Bad Lauterberg, Bad Lauterberg im Harz, Germany,

<sup>2</sup>Department of Biomedical Sciences, Panum Institute, Univ. of Copenhagen, Denmark.

**Background and aims:** Typically, intact GLP-1 concentrations are 15-20 % of total GLP-1 concentrations, but DPP-4 inhibitor treatment only increases intact GLP-1 concentrations by 2-3fold. This discrepancy can either be explained by other interferences (e.g., L cell feedback inhibition mediated by increased concentrations of intact GLP-1) or by an incomplete DPP-4 inhibition in the relevant compartment, e.g. the gut mucosa. The aim of the present

study was to estimate the degree of inhibition of DPP-4 activity in the gut mucosa from concentrations of intact and total GLP-1 (or GIP) determined with and without sitagliptin treatment.

**Materials and methods:** 20 patients with type 2 diabetes (age:  $59 \pm 7$  yrs., duration of diabetes  $5.1 \pm 2.7$  yrs.,  $HbA_{1c}$ :  $7.0 \pm 0.6$  %, BMI  $31.2 \pm 3.2$  kg/m<sup>2</sup>, means  $\pm$  SD, drug-naïve/OAD monotherapy, 6 weeks wash-out) entered a 4-period crossover trial with placebo, sitagliptin (S; 100 mg/d), metformin (M; escalation to 2000 mg/d), and M/S combination (order randomized). On day 5, an oral glucose challenge (75 g) was performed. GLP-1 and GIP (total and intact) were determined by specific RIA.

**Results:** Integrated incremental responses of GLP-1 and GIP are displayed in the table. With placebo treatment, responses of intact GLP-1 were 19.4 % of those for total GLP-1. Sitagliptin treatment induced a 1.71fold (monotherapy;MT) and 1.68fold (metformin background;MB) increment in intact GLP-1, taking the level to 33.3 % of the total GLP-1 under placebo conditions. Total GLP-1 was reduced by sitagliptin treatment (MT: -53.0 %; MB: -46.5 %), indicating L-cell feedback inhibition. Correcting the sitagliptin-induced rise in intact GLP-1 for the degree of feedback inhibition raised intact GLP-1 responses to 70.9 % of the total GLP-1 responses with placebo. With placebo treatment, responses of intact GIP were 30.9 % of those for total GIP. Sitagliptin treatment induced a 1.81fold (MT) and 2.39fold (MB) increment in intact GIP, taking the level to 55.9 % of the total GIP under placebo conditions. Total GIP was reduced by sitagliptin treatment (MT: -28.4 %; MB: -31.3 %), indicating K-cell feedback inhibition. Correcting the sitagliptin-induced rise in intact GIP for the degree of feedback inhibition raised intact GLP-1 responses to 77.8 % of the total GIP responses with placebo.

**Conclusion:** The increment in intact GLP-1 and GIP concentrations with DPP-4 inhibition (sitagliptin treatment) is limited by concurrent L- and K-cell feedback inhibition. A correction for this feedback inhibition does not lead to intact GLP-1 and GIP levels equal to total GLP-1 and GIP, respectively. This may indicate a lesser degree of inhibition of DPP-4 in the relevant compartment(s), e.g. the gut mucosa, than has previously been reported for serum DPP-4 inhibition (> 85 %).

Integrated incremental responses of GLP-1 and GIP (pmol.l<sup>-1</sup>.min) after oral glucose loads (75 g)

Parameter	Experimental conditions				p-values (ANCOVA)		
	Placebo	Sitagliptin	Metformin	Sitagliptin + Metformin	Sitagliptin	Metformin	Interaction
Intact GLP-1	200 $\pm$ 45	342 $\pm$ 89	295 $\pm$ 56	495 $\pm$ 126	0.044	0.14	0.73
Total GLP-1	1025 $\pm$ 164	482 $\pm$ 105	1247 $\pm$ 168	667 $\pm$ 142	< 0.0001	0.10	0.88
Intact GIP	1986 $\pm$ 301	3600 $\pm$ 362	2082 $\pm$ 315	4978 $\pm$ 436	< 0.0001	0.029	0.43
Total GIP	6437 $\pm$ 695	4608 $\pm$ 514	6813 $\pm$ 838	4681 $\pm$ 539	0.0001	0.65	0.76

ANCOVA: Values determined under placebo were used as covariate

Clinical Trial Registration Number: 2008-001663-11

Supported by: Merck Research Labs, Rahway, NJ

## 247

### Effects of a xylose preload, with or without sitagliptin, on gastric emptying and postprandial glycaemia in type 2 diabetes

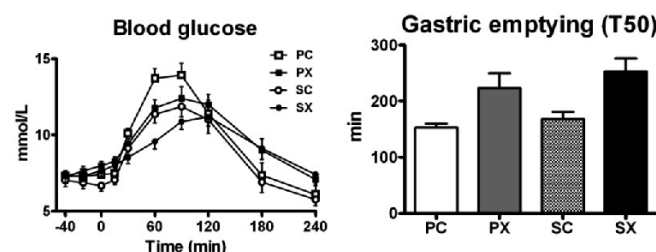
T. Wu, B.R. Zhao, M. Horowitz, K.L. Jones, C.K. Rayner;  
Discipline of Medicine, University of Adelaide, Australia.

**Background and aims:** Fat and protein 'preloads' taken before a meal can reduce postprandial glycaemic excursions in health and type 2 diabetes, at least in part, by slowing gastric emptying (GE) and stimulating GLP-1, but entail additional energy intake. It is not known whether the combination of a 'preload' with a dipeptidyl peptidase-4 (DPP-4) inhibitor would improve efficacy. We have evaluated the effects of a xylose preload, which yields relatively low energy, with or without sitagliptin, on postprandial glycaemia and GE in type 2 diabetes.

**Materials and methods:** 10 type 2 patients were studied on 4 occasions each in randomised, double-blinded, fashion. Subjects took either 100 mg sitagliptin or placebo the night before each study (~2200h). At 0830h after an overnight fast, they drank a 200 mL 'preload', containing either 50 g xylose, or 80 mg sucralose (control of equivalent sweetness) (i.e. the four treatments were sitagliptin + xylose (SX), sitagliptin + control (SC), placebo + xylose (PX), and placebo + control (PC)). 40min later, they ate a mashed potato meal labeled with 100  $\mu$ g <sup>13</sup>C-octanoic acid. Blood glucose and GE (breath test) were evaluated for 4h. Data are mean  $\pm$  SEM.

**Results:** Both the peak and range (i.e. maximum minus minimum value) of postprandial blood glucose concentrations were highest after PC (peak  $14.3 \pm 0.7$  mmol/L, range  $8.6 \pm 0.6$  mmol/L), and lowest after SX (peak  $11.9 \pm 0.5$  mmol/L, range  $5.4 \pm 0.5$  mmol/L) ( $P < 0.001$  for each), while there was no difference between SC (peak  $12.8 \pm 0.6$  mmol/L, range  $7.1 \pm 0.6$  mmol/L) and PX (peak  $12.8 \pm 0.7$  mmol/L, range  $6.3 \pm 0.6$  mmol/L). GE was slower after xylose than control (half-emptying time (T50) for PX  $223 \pm 28$  min and SX  $253 \pm 25$  min vs PC  $153 \pm 7$  min and SC  $167 \pm 13$  min,  $P < 0.0001$ ), without any effect of sitagliptin. The postprandial glycaemic range was inversely associated with the T50 ( $r = -0.50$ ,  $P = 0.001$ ).

**Conclusion:** The combination of a DPP-4 inhibitor with a low nutrient 'preload' represents a promising strategy for the management of type 2 diabetes.



Clinical Trial Registration Number: 2011/0638

Supported by: NHMRC grant

## 248

### Reduced incretin effect in truncally vagotomised subjects

A. Plamboeck<sup>1,2</sup>, S. Veedfald<sup>1,3</sup>, C.F. Deacon<sup>2</sup>, A. Wettergren<sup>3</sup>, L.B. Svendsen<sup>3</sup>, S. Meisner<sup>4</sup>, C. Hovendahl<sup>5</sup>, J.J. Holst<sup>2</sup>, E.K. Knop<sup>1</sup>, T. Vilsbøll<sup>1</sup>;

<sup>1</sup>Diabetes Research Division, Department of internal medicine, Gentofte Hospital, University of Copenhagen, Hellerup, <sup>2</sup>The Novo Nordisk Foundation Center for Basic Metabolic Research, Department of Biomedical Sciences, Panum Institute, University of Copenhagen, <sup>3</sup>Department of Surgical Gastroenterology, and Liver Transplantation, Rigshospitalet, University of Copenhagen, <sup>4</sup>Department of Surgical Gastroenterology, Bispebjerg Hospital, University of Copenhagen, <sup>5</sup>Department of Surgical Gastroenterology, Odense University Hospital, University of Southern Denmark, Denmark.

**Background and aims:** Rapid degradation of glucagon-like peptide-1 (GLP-1) by dipeptidyl peptidase 4 suggests that GLP-1 may act locally (through vagal afferents) before being degraded. The aim of the current study was to clarify the role of vagal innervation on the incretin effect.

**Materials and methods:** Ten truncally vagotomised subjects (due to duodenal ulcer) with pyloroplasty (68 $\pm$ 2 years; fasting plasma glucose (FPG): 6.0 $\pm$ 0.2 mmol/l), 10 subjects treated for oesophageal cancer with cardia resection including truncal vagotomy/pyloroplasty (65 $\pm$ 2 years; FPG: 5.8 $\pm$ 0.3 mmol/l) and 10 control subjects (67 $\pm$ 1 years; FPG: 5.3 $\pm$ 0.1 mmol/l) underwent 4h 50 g-OGTT and isoglycaemic glucose infusion (IIGI).

**Results:** Isoglycaemia during the oral and iv glucose administration was obtained. Peak plasma GLP-1 levels were ~5-fold higher (109 $\pm$ 19 and 140 $\pm$ 40 vs. 24 $\pm$ 3 pmol/l,  $p < 0.02$ ) and gastric emptying faster (paracetamol  $T_{max}$ : 38 $\pm$ 7 and 33 $\pm$ 7 vs. 77 $\pm$ 8 min,  $p < 0.002$ ) in vagotomised subjects after OGTT compared to controls. Glucagon concentrations were suppressed similarly after both iv (total area under the curve (tAUC): 1184 $\pm$ 147 4h $\times$ pmol/l) and oral glucose (1292 $\pm$ 180 4h $\times$ pmol/l,  $p = NS$ ) in controls, whereas vagotomised subjects suppressed glucagon during IIGI and exhibited hyperglucagonemic responses following OGTT (vagotomy: 1374 $\pm$ 174 vs. 1891 $\pm$ 276 4h $\times$ pmol/l,  $p < 0.05$ ; cardia resection: 1125 $\pm$ 139 vs. 1608 $\pm$ 237 4h $\times$ pmol/l,  $p < 0.05$ ). The incretin effect was reduced in the vagotomised groups (48 $\pm$ 7% (vagotomy), 45 $\pm$ 5% (cardia resection,  $p = NS$ ) compared to the controls (64 $\pm$ 6%,  $p < 0.05$ ).

**Conclusion:** The incretin effect was diminished in vagotomised subjects despite 5-fold higher OGTT-induced GLP-1 levels. Furthermore, vagotomised subjects had inappropriate glucagon responses after an oral glucose load. These findings may indicate that an intact vagal innervation is important for the effect of GLP-1 and thereby maintenance of normal glucose homeostasis.

Clinical Trial Registration Number: H-A-2009-060

Supported by: EFSD/Novo Nordisk grant

## OP 45 Diabetes and depression

249

### Psychological impact of diabetes and clinical outcomes: do women with type 2 diabetes require more intense and specific support?

A. Nicolucci<sup>1</sup>, S. Gentile<sup>2</sup>, G. Lucisano<sup>1</sup>, G. Marra<sup>3</sup>, F. Pellegrini<sup>1</sup>, M.C. Rossi<sup>1</sup>, G. Vespasiani<sup>4</sup>, BENCH-D Study Group;

<sup>1</sup>Consorzio Mario Negri Sud, S. Maria Imbaro <sup>2</sup>Federico II University, Naples, <sup>3</sup>NovoNordisk Italia, Rome, <sup>4</sup>Madonna del Soccorso Hospital, S. Benedetto del Tronto, Italy.

**Background and aims:** Patient-centered outcomes are considered of primary importance in the management of chronic conditions. We evaluated the impact of gender on psychological well-being and diabetes related stress in type 2 diabetes (T2DM) patients.

**Materials and methods:** Overall, 28 Italian diabetes outpatient clinics identified a random sample of patients who filled in the WHO-5 well-being index and the Problem Areas in Diabetes (PAID-5) questionnaires. The WHO-5 score ranges between 0 and 100; a score  $\leq 28$  is an indication for testing for depression; the PAID-5 score ranges between 0 and 100, and higher scores indicate poorer adaptation to diabetes. Additional questionnaires were: Diabetes Empowerment Scale (DES), Barriers to Medications (BM), and Global Satisfaction for Diabetes Treatment (GSDT). Clinical data were extracted from computerized medical records. Multiple logistic regression models adjusted for socio-demographic and clinical characteristics were applied to investigate the likelihood to have a WHO-5 or a PAID-5 score in the worst quartile.

**Results:** Overall, 2434 patients were evaluated (mean age  $65.0 \pm 10.2$  years, diabetes duration  $13.8 \pm 15.2$  years, 59.9% males, 48.6% treated with OHA, 25.3% treated with insulin+OHA, 24.3% treated with insulin). Female patients showed lower levels of psychological well-being than males (WHO-5 score of  $50.8 \pm 23.2$  vs.  $62.0 \pm 21.6$ ;  $p < 0.001$ ) and worse adaptation to diabetes (PAID-5 score of  $49.0 \pm 29.0$  vs.  $41.0 \pm 29.0$ ;  $p < 0.001$ ). After adjusting for socio-demographic and clinical characteristics, the likelihood of having a WHO-5 score in the lowest quartile was two times higher in women than in men (OR=1.99; 95%CI 1.51–2.63). Similarly, the likelihood of having a PAID-5 score in the worst quartile was 57% higher in women than in men (OR=1.57; 95%CI 1.17–2.10). Significantly worse levels of PAID-5 score were found in women not reaching the HbA1c target  $\leq 7\%$  as compared with those reaching the target ( $52.9 \pm 27.4$  vs.  $45.4 \pm 27.5$ ;  $p = 0.0003$ ); no association between PAID-5 score and HbA1c target was found in men. Higher levels of diabetes related stress and lower levels of psychological well-being were associated with lower levels of satisfaction with treatment (GSDT) ( $R = -0.30$ ;  $p < 0.0001$  for PAID-5 and  $R = 0.22$ ;  $p < 0.0001$  for WHO-5), lower levels of diabetes empowerment (DES) ( $R = -0.25$ ;  $p < 0.0001$  for PAID-5 and  $R = 0.28$ ;  $p < 0.0001$  for WHO-5), and higher levels of perception of barriers to medication (BM) ( $R = 0.22$ ;  $p < 0.0001$  for PAID-5 and  $R = -0.21$ ;  $p < 0.0001$  for WHO-5).

**Conclusion:** Female gender is associated with poorer psychological well-being and adaptation to diabetes, which in turn are associated with patient attitudes and the achievement of the desired target. These findings suggest the need for enhanced psychological support and specific education in women with T2DM.

Supported by: NovoNordisk Italia

250

### Lifetime depression and type 2 diabetes: the Fremantle diabetes study phase II

W.A. Davis<sup>1</sup>, T.M.E. Davis<sup>1</sup>, S.E. Starkstein<sup>2</sup>, D.G. Bruce<sup>1</sup>;

<sup>1</sup>School of Medicine and Pharmacology, <sup>2</sup>School of Psychiatry & Clinical Neurosciences, University of Western Australia, Fremantle, Australia.

**Background and aims:** There is a bi-directional association between depression and type 2 diabetes (T2DM). Depression occurring after diabetes diagnosis may reflect the burden of disease and/or its complications and have different causes, clinical features and management than depressive illness that develops before diagnosis. Our aim was to compare the characteristics of T2DM in patients in which depression pre-dated diabetes (Group 1) with those of patients developing depression after diabetes diagnosis (Group 2) in a large representative cohort.

**Materials and methods:** The longitudinal observational Fremantle Diabetes Study Phase II recruited 1,551 patients with T2DM from an urban Australian

community between 2008 and 2011. The 9-item Patient Health Questionnaire (PHQ9) was part of detailed baseline assessment. The PHQ9 was modified to assess lifetime depression and age at first depressive episode (PHQ9 Lifetime), and was validated against DSM-IV criteria in a sub-group of patients. The standard PHQ9 questionnaire, which defined the presence and severity of any (major or minor) current depression, was completed by 1443 (93%) and the PHQ9 lifetime questionnaire by 1,384 (89%).

**Results:** In the 1,384 with complete data (mean age 66.2 years; 52.9% males), 288 (20.8%) had a history of depression of whom 193 (67.0%) were in Group 1 and 95 (33.0%) in Group 2. The onset of first depression occurred a median of 13.6 years before diabetes diagnosis at a mean age of 37.3 years in Group 1 and a median of 7.1 years after diabetes diagnosis at a mean 58.4 years of age in Group 2. Compared with Group 1 participants, those in Group 2 were significantly more centrally obese, younger at diabetes diagnosis, and had longer diabetes duration, worse glycaemic control, more intensive blood glucose-lowering treatment, a greater burden of microvascular complications and a higher prevalence of self-reported intermittent claudication ( $P \leq 0.05$ ). Similar proportions of participants in the two groups were currently depressed (24.9% vs 34.7% in Groups 1 and 2, respectively,  $P = 0.10$ ) and taking antidepressant medications (26.4% vs 31.6%,  $P = 0.40$ ), but the percentage of Group 1 patients with controlled depression (no current PHQ9 depressive symptoms on treatment) was significantly greater than that in Group 2 (41.5% vs 23.3%,  $P = 0.050$ ). The prevalence of untreated depression was similar (37.8% vs 30.2%,  $P = 0.44$ ). Compared with participants without a lifetime history of depression, both Groups reported significantly lower compliance with taking diabetes medications (both  $P \leq 0.01$ ).

**Conclusion:** The lifetime risk of depression in patients with T2DM is very high. Depression precedes the development of diabetes in most cases. Depression occurring after diabetes diagnosis is associated with an earlier onset and longer duration of diabetes, more complex treatment and an increased risk of vascular complications. These patients appear to be relatively resistant to antidepressive therapy, suggesting a distinct underlying pathophysiology. The temporal relationship between depression and diabetes may have important implications for clinical management.

Supported by: NHMRC grant 513781

251

### The reach of screening for and treating of subsyndromal depression in type 2 diabetic patients

M. Pibernik-Okanovic<sup>1</sup>, M. Sekerija<sup>2</sup>, D. Ajdukovic<sup>1</sup>, N. Hermanns<sup>3</sup>;

<sup>1</sup>Vuk Vrhovac University Clinic for Diabetes, <sup>2</sup>Croatian National Institute of Public Health, Zagreb, Croatia, <sup>3</sup>Research Institute of the Diabetes Academy Mergentheim (FIDAM), Bad Mergentheim, Germany.

**Background and aims:** Elevated depressive symptoms, common in diabetic patients, have suboptimal recognition and treatment rates. This study aimed to assess the reach of depression screening followed by treatment programmes for subsyndromal depression in a large sample of type 2 diabetic patients, and determine predictors of patients' participation in treatment.

**Materials and methods:** A sample of 4196 type 2 patients (44% female, aged  $56 \pm 8$  yrs, educated for  $12 \pm 3$  yrs, with diabetes duration of  $10 \pm 7$  yrs, BMI of  $30 \pm 5$  kg/m<sup>2</sup> and HbA1C of  $7.2 \pm 1.4$ ) was screened for depressive symptoms. They were sent the two-item Patient Health Questionnaire-Depression scale (PHQ-2) accompanied by an additional question inquiring into the need to receive professional help in mood-related issues. Disease-related data (BMI, diabetes duration, HbA1C, total cholesterol, LDL, HDL, triglycerides and creatinine) were extracted from the electronic files. Patients with positive screening results were phoned to collect personal data (professional, economic and family status, and self-reported acute and chronic stress) and to assess the severity of depressive symptoms by a structured clinical interview (SCID-I). Patients classified as having subclinical depressive symptoms were invited to be randomized to a psychoeducational, physical exercise or diabetes re-education treatment group. The reach of the screening procedure was evaluated based on the total response rate, the proportion of positive depression screenings, and the proportion of eligible patients who entered the treatment programmes. Demographic and disease-related characteristics were explored in patients with elevated depressive symptoms vs. depression-free patients by using chi-square and t-tests. Multivariate logistic regression analysis was used to determine which demographic and disease-related factors differentiated between individuals who entered the treatment and those who declined to participate.

**Results:** Of the 34% of patients who returned the questionnaire ( $n = 1420$ ), 40% reported elevated depressive symptoms and a need for professional help



( $n=581$ ). One-half (48%,  $n=282$ ) were considered eligible for treatments, with 191 (68%) entering the treatment programmes and 91 declining to do so. Responsiveness to screening, reporting depressive symptoms and expressing a need for help were comparable in female and male respondents (all  $p$ 's  $>0.05$ ). Treatment participants in comparison with non-participants had lower BMI ( $29 \text{ kg/m}^2 \pm 4.3$  vs.  $31 \text{ kg/m}^2 \pm 4.9$ ,  $p=0.02$ ) and triglycerides ( $1.9 \text{ mmol/L} \pm 0.9$  vs.  $2.4 \text{ mmol/L} \pm 2.9$ ,  $p=0.02$ ). Female gender reached borderline significance (chi-square=3.62,  $p=0.057$ ), and no differences were detected in other personal and disease-related variables. In multivariate analysis, gender (Wald  $\chi^2=3.84$ ,  $p=0.05$ ), education (Wald  $\chi^2=3.81$ ,  $p=0.05$ ) and BMI (Wald  $\chi^2=5.96$ ,  $p=0.01$ ) were shown to predict participation in treatment.

**Conclusion:** One-third (34%) of type 2 patients responded to postal screening for depression, 40% of them reporting elevated symptoms and a need for professional help. Two-thirds (68%) of eligible individuals were willing to enter treatment programmes. Women, better-educated persons and those with lower BMI were more likely to agree to participate in treatment programmes for subsyndromal depression.

Clinical Trial Registration Number: ISRCTN05673017

Supported by: EFSD New Horizons grant

## 252

### Effects of physical activity on quality of life, depressive symptoms and emotional well-being in type 2 diabetes mellitus: a systematic review

M.M.P. van der Heijden, F.E.P. van Dooren, V.J.M. Pop, F. Pouwer;  
Department of Medical Psychology and Neuropsychology, Tilburg University, Netherlands.

**Background and aims:** Achieving and/or maintaining an appropriate level of physical activity is an important goal in type 2 diabetes management, since physical inactivity is known to be one of the major risk factors for type 2 diabetes related complications. Systematic reviews and meta-analysis regarding the effects of physical activity in type 2 diabetes, so far mainly focused on biomedical outcomes. However, type 2 diabetes is also associated with psychological problems, concerning -but not limited to- depressive symptoms, decreased quality of life and decreased emotional well-being, which appear also to be related to the level of physical activity. The aim of this study is to conduct a systematic review in order to assess the effects of physical activity on depressive symptoms, quality of life and emotional well-being in type 2 diabetes patients.

**Materials and methods:** MEDLINE, PSYCHINFO, EMBASE and Clinicaltrials.gov databases were searched. The search comprised the terms type 2 diabetes mellitus, physical activity, randomized controlled trial, quality of life, depression, well-being, and related entry terms. Randomized controlled trials of at least 4 weeks' duration in type 2 diabetes patients, that evaluated the effect of a physical activity intervention on depressive symptoms and/or quality of life and/or emotional well-being compared to a control group with no or minimal intervention, were included. Risk of bias was assessed in accordance with the PRISMA statement.

**Results:** Of 1224 retrieved articles, 20 RCTs were included with a total of 1086 participants. 10 RCTs contained an aerobic activity, 5 RCTs a resistance activity and 10 RCTs a combined activity intervention. Intervention frequencies varied between 1x/week to 5x/week, session durations varied between 10 and 60 minutes. Program duration varied between 4 weeks and 6 months. Quality of life was assessed in 16 studies. Between group comparisons showed no significant results for aerobic activity and mixed results for resistance and combined interventions on quality of life. Depressive symptoms were assessed in 4 studies of which only one resistance intervention showed significant results. Emotional well-being was evaluated in 8 studies, also showing mixed results. Adequate random sequence generation was performed in 16 studies, adequate allocation concealment in 8 studies (unclear in 12 studies), blinding of outcome assessment was performed in 3 studies (unclear in 17 studies), losses and exclusions were adequately described in 16 studies and intention to treat analysis was performed in 5 studies (unclear in 7 studies).

**Conclusion:** Physical activity interventions that consist of resistance exercise or combined exercise may improve quality of life in patients with type 2 diabetes, although the results were mixed. Effects of physical activity interventions on depressive symptoms and emotional well-being are unclear. The large heterogeneity of the current interventions, heterogeneity of tests used to evaluate these psychological parameters and the limited number of studies evaluating depressive symptoms and well-being, limited the ability to draw conclusions. There is a need for higher quality RCTs evaluating the effect of physical activity interventions on psychological parameters in type 2 diabetes patients.

## OP 46 Foot ulceration: Can we do better?

### 253

#### Decreasing incidence of foot ulcers among patients with type 1 and type 2 diabetes

A. Rasmussen, T. Almdal, K.E. Nielsen, U.B. Christensen, A.A. Nielsen, P. Holstein;

Steno Diabetes Center, Gentofte, Denmark.

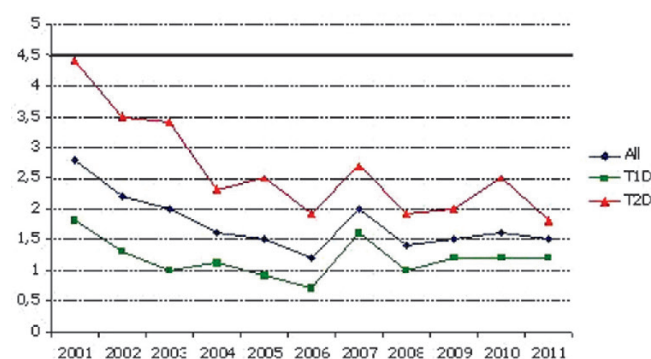
**Background and aims:** Foot ulcers are costly complication among diabetes patients. Figures of incidence of foot ulcers varies and there are only limited information in relation the change of incidence over time. The aim was to study the development in incidence of first foot ulcers in two large cohort of type 1 and type 2 diabetes followed for 10 years.

**Methods:** In Denmark all patients with type 1 diabetes (T1D) are followed lifelong in hospitals outpatients clinics, patients with type 2 diabetes (T2D) are only followed in hospitals clinics if they have advanced disease. Our clinic is a specialized diabetes clinic and is an integrated part of the public health care system. App 3500 patients with T1D and app 2000 patients with complicated T2D are followed. All patients are offered yearly feet inspection and education including determination of vibration threshold. Patients with high risk and/or abnormal vibration threshold ( $>40 \text{ mV}$ ) are seen by a chiropodist for instruction. When a foot ulcer is observed the patients is seen by the chiropodist immediately and if necessary a specialist in orthopedic surgery within at max 1 week. At the initial visit it is determined whether etiology of the ulcer is predominantly neuropathic or ischemic. All information in relation to the patients are housed in an electronic patient's record system. We determined the yearly incidence of the 1st registered foot ulcer among all T1D and T2D patients followed regularly in the clinic. Clinical characteristics of patients developing an incident foot ulcer in 2002 and 03 and 2010 and 11 are given.

**Results:** Changes in incidence over a 10 year period among patients with type 1 and type 2 diabetes appear in the figure. Among patients with T1D and first ulcers diagnosed in 2002 and 03,  $N=71$ , compare to those with first ulcer diagnosed in 2010 and 11,  $N=85$ , mean HbA1c were 9,2/8,8%, in T2D corresponding figures for 2002 and 03,  $N=135$ , and 2010 and 2011,  $N=92$ , mean HbA1c were 8,3%/8,3%. Proportion with diabetic retinopathy / microalbuminuria / macroalbuminuria in 2002 and 03 among in T1D patients were 72/29/28% and in 2010 and 11 62/ 39 /19%. The corresponding figures among T2D patients were 2002 and 03 58 / 32 / 21% and in 2010 and 2011 64/ 38 /15. Patients with T1D had a mean disease duration at time of first foot ulcer of 34,5 years in 2002 and 03 compared to 35 years in 2010 and 1. The corresponding figures for T2D patients were in 2002 and 03 15 years and in 2010 and 11 17 years.

**Conclusion:** The present study demonstrate that the incidence of 1 st foot ulcers have decreased in both patients with T1D and T2D, most pronounced in T2D. Patients developing ulcers have long disease duration and a high proportion of diabetes complications.

Incidence of first footulcer pr 100 patients pr patientyear



## 254

**Circulating pro-angiogenic cell number and growth factor profile may be altered in type 2 diabetes with/without diabetic foot syndrome**W.N. Nowak<sup>1</sup>, S. Borys<sup>2</sup>, K. Kusinska<sup>1</sup>, P. Witek<sup>2</sup>, T. Koblik<sup>2</sup>, A. Jozkowicz<sup>1</sup>, M. Malecki<sup>2</sup>, J. Dulak<sup>1</sup>;<sup>1</sup>Department of Medical Biotechnology, Jagiellonian University,<sup>2</sup>Department of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland.

**Background and aims:** Type 2 diabetes mellitus (T2DM) is often complicated by diabetic foot syndrome (DFS). DFS pathogenesis may be associated not only with metabolic abnormalities, but also with alterations in stem and progenitor cell mobilization as well as with growth factor levels. It has been shown that both the functionality and the number of endothelial progenitor cells circulating in peripheral blood (PB) are altered in diabetic patients. However, some other stem cell populations potentially involved in wound healing and regeneration have never been examined in T2DM. Here, we analyze several populations of circulating proangiogenic progenitor cells (PACs) and growth factors in T2DM patients with/without different forms of DFS.

**Materials and methods:** We examined patients with four different forms of DFU: a) infected ulceration (DFU-I, n=10), b) non-infected ulceration (DFU, n=11), c) healed ulceration (DFU-H, n=6), and d) Charcot peripheral osteoneuropathy (ChPON, n=10). In addition, we included 17 healthy controls (H) and 10 T2DM patients without DFS. We used flow cytometry to evaluate the presence of the following stem cell populations based on their surface markers: CD45dimCD31+CD133+ and CD45dimCD31+CD34+KDR+ endothelial progenitor cells (EPCs), CD45-CD105+STRO-1+ and CD45-CD29+CD90+ mesenchymal stem cells (MSC), Lin-CD45+CD133+ hematopoietic stem cells (HSC), and Lin-CD45-CD133+ stem cells (SC). Furthermore, we evaluated serum levels of SCF, LIF, TPO, EGF, FGF-2, Flt3-L, G-CSF, TNF $\alpha$ , VEGF, SDF1 $\alpha$ + $\beta$  using Milliplex FlexMap 3D.

**Results:** Numbers of circulating CD45dimCD31+CD133+ were significantly decreased in diabetic patients without (but not with) foot ulceration (T2DM, ChPON) in comparison to healthy controls (T2DM: 584 $\pm$ 85, ChPON: 548 $\pm$ 64 vs. H: 872 $\pm$ 93 cells/1ml of peripheral blood,  $p < 0.05$ ). Similarly, the CD45-CD29+CD90+ MSC population was decreased in patients with T2DM and ChPON foot ulceration (T2DM: 47 $\pm$ 6, ChPON: 51 $\pm$ 18 vs. H: 156 $\pm$ 59 cells/1ml of PB,  $p < 0.05$ ). Number of circulating Lin-CD45-CD133+ SC was decreased ( $p < 0.001$ ) in DFU-I (10 $\pm$ 3 cells/1ml of PB) and ChPON (13 $\pm$ 5 cells/1ml of PB) groups in comparison to the T2DM group (39 $\pm$ 7 cells/1ml of PB). Patients with T2DM had significantly lower levels of serum EGF than healthy controls (T2DM 7.49 $\pm$ 2.16 vs. H: 81.10 $\pm$ 22.28, pg/ml  $p < 0.05$ ). Interestingly, the level of EGF in the group with healed foot ulcers (DFU-H: 93.45 $\pm$ 50.14 pg/ml) was comparable to that of healthy controls. Furthermore, the level of G-CSF was significantly higher in DFU (286.62 $\pm$ 63.65 pg/ml) than in the healthy group (77.15 $\pm$ 13.09 pg/ml;  $p < 0.05$ ).

**Conclusion:** Patients with T2DM and different forms of DFS have a decreased number of circulating pro-angiogenic progenitor cells. In addition, T2DM may be associated with a changed serum growth factor profile. Altogether, some of these factors can contribute to the pathogenesis of T2DM complications such as different forms of DFS.

Supported by: EU Structural Fund POIG.01.01.02-00-109/09

## 255

**Relation between angiogenic cytokines and clinical effect of stem cell therapy in diabetic patients with no-option critical limb ischaemia**M. Dubsky<sup>1</sup>, A. Jirkovská<sup>1</sup>, L. Pagacová<sup>2</sup>, R. Bem<sup>1</sup>, V. Fejfarová<sup>1</sup>, B. Sixta<sup>3</sup>, V. Wosková<sup>1</sup>, J. Skibová<sup>1</sup>, S. Langkramer<sup>4</sup>, E. Sykova<sup>4</sup>;<sup>1</sup>Diabetes Centre, Institute for Clinical and Experimental Medicine,Prague, <sup>2</sup>Autotransfusion Unit, Institute for Clinical and ExperimentalMedicine, Prague, <sup>3</sup>Clinic of Transplant Surgery, Institute for Clinical and Experimental Medicine, <sup>4</sup>Institute of Experimental Medicine, Czech Academy of Science, Prague, Czech Republic.

**Background and aims:** The effect of angiogenic cytokines during the stem cell therapy is probably paracrine, but the exact role of serum levels of these cytokines in assessment of clinical effect and systemic vasculogenesis remains unclear. The release of pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), Angiopoietin-1 (Ang-1), Platelets-derived factors (PDGF-AA and PDGF-BB) and decrease of endogenous inhibitor of angiogenesis (Endostatin) can reflect both local and possibly systemic vasculogenesis after stem cell treatment. The aim

of our study was to assess the levels of angiogenic cytokines and their relation to clinical effect evaluated by changes of transcutaneous oxygen tension (TcPO<sub>2</sub>) in patients with diabetic foot ulcers (DFU) and no-option critical limb ischemia (NO-CLI).

**Materials and methods:** Systemic angiogenesis was evaluated by serum levels of VEGF, b-FGF, Ang-1, Endostatin, PDGF-AA and PDGF-BB after 1, 6 and 30 days from treatment and visualization was assessed by eye fundus examination before and 6 months after therapy. Clinical effect was evaluated by changes of TcPO<sub>2</sub> after 6 months. We included twenty five patients with NO-CLI (defined by TcPO<sub>2</sub> < 30 mm Hg) and DFU (mean age 61.9  $\pm$  9.9 years, mean diabetes duration 21.5  $\pm$  10.6 years) treated in our foot clinic from January 2008 to September 2011. Stem cell treatment was performed by two methods - bone marrow mononuclear cells (14 patients) or peripheral blood progenitor cells obtained by apheresis of peripheral blood stimulated by granulocyte-colony stimulating factor (11 patients). Cell suspension was concentrated, centrifuged and injected into the muscles of the affected limb.

**Results:** No significant correlation between clinical effect and levels of any of tested angiogenic cytokines was observed. Clinical effect measured by increase of TcPO<sub>2</sub> was significantly improved from 15.8  $\pm$  9.7 to 41.8  $\pm$  10.6 mmHg ( $p < 0.001$ ) after 6 months. We did not observe any significant increase of serum levels of pro-angiogenic cytokines in any of the follow-up control visits up to 30 days from procedure. PDGF-AA and PDGF-BB decreased significantly after 30 days ( $p = 0.034$  and  $p = 0.023$ , respectively). Levels of angiogenic inhibitor Endostatin increased significantly after 1 day ( $p = 0.029$ ) and 30 days ( $p = 0.0003$ ). No changes in eye fundus examination after 6 months from the treatment were seen.

**Conclusion:** Our study showed no relation between serum levels of angiogenic cytokines and clinical effect of stem cell therapy measured by TcPO<sub>2</sub>, but the effectiveness of this procedure was confirmed by significant increase of TcPO<sub>2</sub> in patients with NO-CLI. No increase of serum levels of pro-angiogenic cytokines during first month and no changes on eye fundus after 6 months may indicate safety of autologous stem cell treatment in terms of systemic angiogenesis.

Supported by: GAUK 362311 and MZO 00023001

## 256

**Does negative wound pressure therapy have a similar acute antimicrobial effect as maggot and ozone therapy on diabetic foot ulcer infection?**

R. Bem, A. Jirkovská, M. Dubsky, V. Fejfarová, V. Wosková, L. Rezaninová, J. Skibová;

Diabetes Centre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

**Background and aims:** Previous studies showed that maggot debridement therapy (MDT) acutely eliminated most of the bacterial strains in patients with infected diabetic foot ulcers (DFU). Ozone is well known antimicrobial agent with broad-spectrum of medical indications. Negative wound pressure therapy (NWPT) was also reported as a useful method, which may improve infection resolution and wound healing. The aim of our study was to compare the acute antimicrobial effect of ozone therapy, MDT and NWPT with standard local therapy in patients with infected DFU.

**Materials and methods:** 120 patients with infected DFU (Texas 2-3; B and D) hospitalized in our Diabetes Department were enrolled in the present study between January 2010 and February 2012. All patients were treated with parenteral antibiotics appropriate to microbial findings and standard therapy (offloading, surgical intervention, etc.). In addition to standard treatment, 30 patients were treated by gaseous ozone once daily (ozone group), 30 patients by sterile free-range larvae of the green bottle fly *Lucilia sericata* (MDT group) and 30 patients by NWPT (NWPT group). 30 patients received standard local treatment, mainly disinfectant solutions (Controls). There was no significant difference between groups in age, gender, type and duration of diabetes, diabetes control, C-reactive protein, Texas score and presence of osteomyelitis. Swabs or tissue specimens for culture were taken from deep structures of the wound after debridement immediately before and after intervention or in comparable time in Controls. Groups did not differ in the mean therapy duration (ozone 3.3 $\pm$ 1 vs. maggots 3.6 $\pm$ 0.7 vs. NWPT - first dressing 3.4 $\pm$ 0.7 vs. Controls 3.8 $\pm$ 0.6 days; NS). The individual bacterial species in positive swabs were determined by standard microbiological methods.

**Results:** There were no significant differences between groups in the numbers of bacteria strains per specimen before treatment. After the treatment, significant reductions in the numbers of bacteria strains in ozone group (before 2.3 $\pm$ 1.1 vs. after 0.7 $\pm$ 0.7;  $p < 0.0001$ ), in MDT group (2.6 $\pm$ 0.9 vs. 0.7 $\pm$ 0.5;  $p < 0.0001$ ) and in NWPT group (2.2 $\pm$ 0.9 vs. 1.1 $\pm$ 0.9;  $p < 0.0001$ ) were seen, but

there was no significant reduction in Controls ( $2.2 \pm 0.9$  vs.  $1.9 \pm 0.6$ ). All study methods significantly reduced the number of bacteria strains in comparison with Controls (all  $p < 0.01$ ). NWPT reduced the number of bacteria strains significantly less than maggots ( $p < 0.01$ ) and ozone ( $p < 0.05$ ). Ozone was effective especially against *Enterococcus* and *Staphylococcus* sp. (both  $p < 0.05$ ), MDT against *Enterococcus* sp., *Escherichia coli* and *Proteus* sp. (all  $p < 0.05$ ) and NWPT against *Streptococcus* sp. ( $p < 0.05$ ).

**Conclusion:** Our study demonstrated that both ozone therapy or MDT and NWPT added to standard treatment may help influence polymicrobial infection in the DFU in comparison with standard local therapy. NWPT was slightly less effective than both maggot and ozone therapy.

Supported by: MZO 00023001

## OP 47 Tuning into the rhythm of clock genes in diabetes

257

**The effects of adiponectin on free-running locomotor activity rhythm and circadian expression of core clock genes in model mice for metabolic syndrome**

K. Yamada, T. Hashinaga, N. Wada, S. Otabe, H. Nakayama, Y. Tajiri; Endocrinology and Metabolism, Kurume University, Japan.

**Background and aims:** Alterations of circadian rhythms through genetic and environmental influences on the molecular clock are pivotal in the pathogenesis of obesity, type 2 diabetes and cardiovascular disease. The disturbed circadian rhythm in obese subjects can be restored at least partly by weight loss with low-calorie diet or bariatric surgery. However, the mechanism by which excess adipose tissue accumulation affects circadian clock is still largely unknown. In this study we assessed the effect of adiponectin on the circadian rhythm disturbances associated with metabolic syndrome using adiponectin-transgenic mice.

**Materials and methods:** We generated a KK/Ta mouse line expressing the human adiponectin transgene in the liver. Although KK/Ta mice had hypoadiponectinemia, serum adiponectin levels of transgenic KK/Ta mice were higher than those of control C57BL/6 mice. Spontaneous locomotor activity under 12-h light/12-h darkness cycle and free-running rhythm under constant darkness were analyzed using digital counters. The expression of core clock genes was measured by quantitative real-time RT-PCR.

**Results:** Locomotor activity of C57BL/6 mice was highest during the beginning of the dark period and low during the light period. Under constant darkness, the length of locomotor activity rhythm of control mice was slightly shorter than 24 h. In KK/Ta mice the peak of locomotor activity was blunted and significant activity was observed during the light period. Furthermore, KK/Ta mice showed shorter average period length of free-running locomotor activity rhythm. However, the transgenic expression of adiponectin in the liver significantly restored the circadian rhythm of locomotor activity and the length of free-running rhythm of KK/Ta mice. In the liver and skeletal muscles from control mice, mRNA levels of *Arntl* and *Cry1* were increased during the dark period, whereas those of *Dbp*, *Cry2*, *Per1* and *Per2* were elevated during the light period. KK/Ta mice exhibited phase advances in circadian rhythms of *Arntl*, *Dbp*, *Cry2* and *Per2* in both tissues. The phase shifts in the liver were corrected in adiponectin-transgenic mice.

**Conclusion:** The restoration of circadian rhythm was not attributable to weight loss, because there was no significant difference in body weight between KK/Ta mice and adiponectin-transgenic KK/Ta mice. Adiponectin has two different signaling pathways: one mediated by peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ) and the other mediated by 5'-AMP-activated protein kinase (AMPK). PPAR- $\alpha$  has been shown to bind to the *Arntl* promoter and up-regulate its expression. On the other hand, AMPK phosphorylates and destabilizes *Cry1*. Furthermore, adiponectin down-regulates TNF- $\alpha$ , which suppresses several clock genes in the liver. Although further studies were required to elucidate the detailed molecular mechanism by which adiponectin modified circadian rhythm of the metabolic syndrome model animal, adiponectin may be a peripheral regulator of the circadian clocks in the brain and peripheral organs, and may be a novel target for the treatment of obesity-associated disorders of circadian rhythms.

258

**Diabetic complications and circadian rest-activity rhythmicity**

M. Kadono, G. Hasegawa, T. Sennmaru, N. Nakamura; Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Japan.

**Background:** Emerging evidence from a variety of studies indicates that circadian rhythmicity is associated with aging process. Diabetes is considered to be a premature aging process. In particular, diabetic complications, neuropathy and angiopathy, are critical factors for the disease prognosis, accelerating patients' physical aging. Therefore, it is possible that the progression of these complications could involve disruption of circadian rhythmicity. In this study, we investigated the association between actigraphic estimates of the rest-activity rhythm and diabetic complications.

**Materials and methods:** Seventy-two outpatients in our diabetes clinic wore an actigraph for consecutive 7 days (men /women 30/42, age  $68.5 \pm 8.2$



yrs, BMI<25kg/m<sup>2</sup>, type2/type1 diabetes 69/3). Patients with depression, dementia, liver cirrhosis, renal failure, blindness, and shift work were excluded. Activity profiles were analyzed using nonparametric variables, including dichotomy indices, interdaily stability (IS), intradaily variability (IV), relative circadian amplitude (RA), nocturnal awakening (fNA (22:00–1:00), INA (1:00–4:00)), and morning activities (MoA). To cluster diabetic clinical characteristics and determine the clusters predicting circadian rhythms disruption, principal factor analysis (PFA) was applied.

**Results:** BMI was positively correlated with IV and INA ( $P=0.006$  and  $.004$ ), and negatively correlated with IS and RA ( $P=0.007$  and  $=0.008$ ), while diabetic duration was associated with IS ( $P=0.01$ ). Patients with progressive diabetic retinopathy had significantly lower RA, compared to those without retinopathy ( $p=0.02$ ). Patients with symptomatic neuropathy had significantly lower IS, higher fNA, and lower MoA ( $P=0.02$ ,  $.03$ , and  $.02$ , respectively). The levels of urinary albumin excretion were positively correlated with IV and INA ( $P=0.005$  and  $=0.004$ ), and negatively correlated with IS and RA ( $P=0.004$  and  $=0.003$ ). The significance remained after adjusting for BMI. Next, PFA identified two factors that explained 24 and 14 % of the variance in the variance in the dataset of diabetic clinical characteristics, respectively. These factors were interpreted as 1) a “duration” factor (DF) with positive loadings of diabetic duration, tripathic parameters, and the history of cardiovascular diseases, and 2) a “metabolic” factor (MF) with positive loadings of BMI, HbA<sub>1c</sub>, TG, and HDL. Multivariate analysis with a model including DF and MF showed that both factors were independent predictors of IV ( $p=0.04$ , and  $.04$ ), and of IS ( $p=0.02$ , and  $.006$ ).

**Conclusion:** Diabetic complications, angiopathy and neuropathy, were associated with disruption of circadian rest–active rhythm. The current results indicate a key common regulator of circadian rhythms and neurovascular function. In addition, metabolic factors and duration factors were independently associated with disruption of circadian rest–activity rhythms. It can be speculated that lifestyles with irregular circadian rhythmicity will cause difficulty metabolic control including weight and daily blood sugar control, and thus hasten the development of these complications, resulting in a vicious cycle. Taken together, chronobiological approach, especially, circadian rhythms entrainment should be considered as a possible therapy for diabetes.

## 259

### Robust circadian clocks are ticking in beta and non-beta cells of human pancreatic islets

C. Dibner<sup>1</sup>, T. Mannic<sup>1</sup>, D. Sage<sup>2</sup>, S. Lemeille<sup>1</sup>, D. Bosco<sup>1,3</sup>, P. Salmon<sup>3</sup>, C. Bauer<sup>3,2</sup>, M. Unser<sup>2</sup>, P. Halban<sup>3</sup>, J. Philippe<sup>1</sup>

<sup>1</sup>Endocrinology, Diabetes and Nutrition, University Hospital of Geneva,

<sup>2</sup>EPFL, Lausanne, <sup>3</sup>University of Geneva, Switzerland.

**Background and aims:** Due to the emerging evidence of the pancreas clock impact on the islet function and on type 2 diabetes development as shown in rodents, we aimed to tackle the circadian clockwork in human islets. The oscillator properties were assessed in the intact islets, and as well as in  $\beta$ -cells.

**Materials and methods:** We established a system for long term bioluminescence recording in cultured human islets, employing lentivector gene delivery.  $\beta$ -cells were stably labeled by rat insulin2 promoter (RIP) fluorescent construct. Single islet/ cell oscillation profiles were measured by combined bioluminescence - fluorescence time lapse microscopy.

**Results:** Human islets exhibited robust self-sustained circadian oscillations of *Bmal1-luciferase* expression at the both populations and single islet levels, with the oscillation period of 23.6 and 23.9 hours respectively. Moreover, endogenous *Bmal1* transcript expression was circadian in synchronized islets over 48 hours, and antiphasic to *Reverba*, *Per2* and *Dbp* transcript circadian profiles. Importantly, single  $\beta$ - and non- $\beta$  cells revealed oscillatory profiles well synchronized with each other.

**Conclusion:** We provide for the first time a compelling evidence for a robust cell autonomous clock ticking in human islets. Moreover,  $\beta$ -cells possess their own clocks oscillating in synchrony with non- $\beta$ -cells in primary human islet cell culture.

Supported by: EFSD/MSD grant

## 260

### An isocaloric high fat diet modulates daily expression profiles of clock genes, LPS response and fat metabolism genes in human monocytes

O. Pivovaro<sup>1,2</sup>, S. Hornemann<sup>1,2</sup>, L. Ye<sup>1,2</sup>, S. Möckel<sup>1</sup>, M. Kruse<sup>1,2</sup>, J. Mazuch<sup>3</sup>, A. Kramer<sup>3</sup>, A. Busjahn<sup>4</sup>, A.F.H. Pfeiffer<sup>1,2</sup>

<sup>1</sup>Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, <sup>2</sup>Department of Endocrinology, Diabetes and Nutrition, Campus Benjamin Franklin, Charité University Medicine, Berlin, <sup>3</sup>Laboratory of Chronobiology, Institute for Medical Immunology, Charité University Medicine, Berlin, <sup>4</sup>HealthTwist GmbH, Berlin, Germany.

**Background and aims:** The circadian clock coordinates various behavioural and physiological processes including feeding, energy metabolism and inflammation response. In turn, metabolic processes feed back onto the circadian clock as shown in recent human and animal studies. However, there is little to no information about the effect of nutrition on circadian mechanisms in humans. To address this, we provided the analysis of daily expression profiles of clock genes, LPS response and fat metabolism genes in human blood monocytes before and after the high-fat diet intervention.

**Materials and methods:** Daily gene expression profiles were determined by real-time PCR in 30 non obese healthy individuals in terms of NUTriGenomics Analysis in Twins (NUGAT) study. Gene expression was measured at three time points (in the morning, in the middle of the day and afternoon) during three investigation days. The blood sampling was carried out before the beginning of the high-fat isocaloric diet (HFD, 45 % kcal from fat) and after one and six weeks of intervention.

**Results:** We demonstrated that clock genes (*PER1*, *PER2*, *PER3*, *BMAL1*, *REV-ERB $\alpha$* , *DBP* and *TEF*) as well as genes contributing to LPS response (*CD14*, *IKB $\alpha$* , *CD180*, *ERK1*, *IL1 $\beta$* , *IL10*, *TNF $\alpha$* , *CCl3*) and fat metabolism (*FASN*, *CPT1a*) exhibited significant daily variation in human monocytes. The HFD induced the increase of the expression of the *Period* genes *PER1*, *PER2* and *PER3* and *TEF* after one and six weeks of intervention ( $p<0.05$ ) and alterations of synchronisation state within clock gene system. Moreover, the HFD effected the expression of LPS response genes *CD14*, *IKB $\alpha$*  and *IL8* and fat metabolism genes *ACOX3* and *IDH3A*. Furthermore, the expression levels of *Period* genes and *TEF* significantly correlated with blood cholesterol levels and with the *ACOX3* and *IDH3A* expression in monocytes.

**Conclusion:** Our results suggest that the consumption of an isocaloric HFD can influence the circadian clock as well as circadian expression of genes contributing to LPS response and fat metabolism in humans already after the short time intervention. This emphasizes the role of nutrition-clock interaction in the regulation of human metabolism and inflammation response.

Supported by: Deutsche Forschungsgemeinschaft (DFG grant Nr. KFO218)

## OP 48 Imaging beta cell mass in vivo

### 261

**In vivo imaging of beta cell mass by [<sup>11</sup>C]5-hydroxy-tryptophan PET**  
**O. Eriksson**<sup>1</sup>, D. Espes<sup>2</sup>, R. Selvaraju<sup>1</sup>, J. Sörensen<sup>3</sup>, M. Lubberink<sup>3</sup>, G. Antoni<sup>3</sup>, L.A. Johansson<sup>3,4</sup>, J. Eriksson<sup>4</sup>, P.-O. Carlsson<sup>2,5</sup>, B. Eriksson<sup>5</sup>, O. Korsgren<sup>6</sup>;  
<sup>1</sup>Preclinical PET Platform, Uppsala, <sup>2</sup>Dept. of Medical Cell Biology, Uppsala, <sup>3</sup>Department of Radiology, Oncology and Radiation Sciences, Uppsala, <sup>4</sup>AstraZeneca, Mölndal, <sup>5</sup>Dept. of Medical Sciences, Uppsala, <sup>6</sup>Dept. of Immunology, Genetics and Pathology, Uppsala, Sweden.

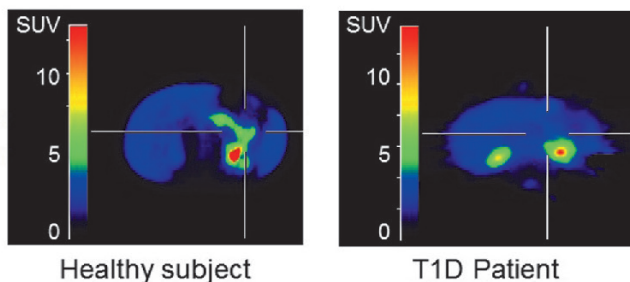
**Background and aims:** The ability to undertake repeated non-invasive monitoring of pancreatic beta cell mass (BCM) in subjects with T1D and T2D would be of immense importance. The PET tracer [<sup>11</sup>C]5-hydroxy-tryptophan ([<sup>11</sup>C]5-HTP) rapidly accumulates in cells using serotonin. As serotonin is present in beta cells, but not in exocrine pancreas, we hypothesized that [<sup>11</sup>C]5-HTP could be used as a biomarker for BCM.

**Materials and methods:** Islet specificity of [<sup>11</sup>C]5-HTP was assessed by IHC staining for serotonin on pancreatic sections from T1D and healthy controls, and by incubation of human islet cell preparations. Control and STZ-treated rats were administered [<sup>11</sup>C]5-HTP and the pancreatic uptake was determined by organ distribution. In vivo specificity was assessed in a non-human primate model. Pancreatic uptake was investigated in healthy controls and subjects with T1D or T2D in clinical studies.

**Results:** IHC staining showed co-localization of serotonin with insulin in healthy pancreas, and serotonin positivity diminished in parallel with insulin in T1D. Incubation of isolated human islets mixed in different ratios with exocrine cells showed strong positive linear correlation ( $R^2 > 0.9$ ,  $p < 0.05$ ) between islet purity and [<sup>11</sup>C]5-HTP uptake. Pancreatic uptake in diabetic rats was decreased (Standard Uptake Value; SUV  $2.6 \pm 0.4$ ,  $p < 0.05$ ) compared to non-diabetic controls (SUV  $5.0 \pm 0.2$ ), and correlated to decrease in beta cell mass as assessed by IHC. PET/CT of non-human primates showed that 80% of the pancreatic uptake was dependent on the serotonin biosynthesis pathway as it could be inhibited by iv administration of carbidopa. Retrospective data in non-diabetic patients showed a distinct pancreatic uptake (SUV  $= 2.9 \pm 0.4$ ), while uptake in two T2D patients was decreased by 33 and 56%, respectively. In one of the patients, repeated [<sup>11</sup>C]5-HTP PET measurements showed gradual reduction in parallel with the progression of T2D from diagnosis to insulin requirement. An ongoing prospective [<sup>11</sup>C]5-HTP PET study demonstrated accumulation of tracer in pancreas of controls ( $n=3$ ), whereas tracer uptake in the pancreas of T1D patients ( $n=2$ ) decreased towards background within 60 min (figure).

**Conclusion:** We show both experimental and clinical results demonstrating the feasibility of [<sup>11</sup>C]5-HTP PET as novel method for quantifying human BCM, allowing monitoring of disease progression as well as evaluation of new therapies.

**Fig.** Distinct uptake of [<sup>11</sup>C]5-HTP in the pancreas of a healthy subject (pancreas in origo; left panel), whereas low uptake is seen in the pancreas of a c-peptide negative T1D patient (right panel). Tracer excreted into urine causes the kidney signal.



Clinical Trial Registration Number: NCT01552811

Supported by: JDRF, Vinnova

### 262

**Multimodality non-invasive imaging of the functional beta cell mass using various formats of the beta cell surface specific monoclonal autoantibody IC2**

**C.-H. Brogren**<sup>1</sup>, I.D. Pedersen<sup>1</sup>, K.L. Jensen<sup>1</sup>, A. Briat<sup>2</sup>, L. Bronsart<sup>3</sup>, M.H. Bachmann<sup>3</sup>, L. Renaut<sup>4</sup>, P. Mondon<sup>4</sup>, W. Petersen<sup>5</sup>, S. Manohar<sup>5</sup>, B. Hesse<sup>6</sup>, A. Kjær<sup>6</sup>, K. Buschard<sup>1</sup>, D. Christiaen<sup>2</sup>, C.H. Contag<sup>3</sup>;  
<sup>1</sup>The Bartholin Institute, Rigshospitalet, Copenhagen, Denmark, <sup>2</sup>Animascope, Grenoble, France, <sup>3</sup>Molecular Biophotonics and Imaging Laboratory, Stanford School of Medicine, Palo Alto, USA, <sup>4</sup>Millegen, Toulouse, France, <sup>5</sup>University of Twente, Enschede, Netherlands, <sup>6</sup>Department of Biomedical Sciences, University of Copenhagen, Denmark.

**Background and aims:** For more than a decade multiple tracers have been used in an attempt to quantitatively and specifically image the  $\beta$ -cell mass (BCM) in vivo. However, none of the drugs and only one of the antibody based tracers applied have shown sufficient specificity and affinity, which is the rat monoclonal autoantibody IC2. This autoantibody raised from a newly diabetic BB-rat has been shown to have a unique specificity for the surface of insulin secreting pancreatic  $\beta$ -cells from multiple animal species and humans. The autoantibody has no diabetogenic or cytotoxic effects in vivo. The first in vivo attempt as biomarker for BCM, but not fully noninvasive, was done by SPECT and <sup>111</sup>In-labelled IC2-IgM. However such a large IgM tracer molecule will not easily be excreted if in excess, so attempts have been made to use either the pepsin fragments F(ab')<sub>2</sub> and Fab, or alternatively recombinant and chimeric formats, which in size span from 50–150 kDa, to enable a faster excretion. The pancreas is high vascularised, which ensure a fast and easy delivery of all tracers formats to targeted islets.

**Materials and methods:** First fully noninvasive imaging trials were done on rats with a 2-head SPECT and <sup>111</sup>In-labelled IC2 as tracer, which were followed up by both rat and mice biodistribution studies using <sup>125</sup>I Iodogen-labeled IC2-IgM, F(ab')<sub>2</sub>, and Fab, two other monoclonal antibodies, K14D10, A2B5, and a rat isotype IgM control. Our second imaging modality uses Gaussian luciferase-IC2-diabody format in noninvasive BLI on mice. The third modality applies gold nanorods labeled with IC2 in animal photoacoustic imaging, and our fourth modality will be near-infrared fluorescence-mediated tomography using Vivotag-680nm and VivoTag-750nm labeled pepsin fragments of F(ab')<sub>2</sub> and Fab plus a newly engineered recombinant chimeric rIC2-hIgG1 format applied in mice BCM imaging.

**Results:** The first fully proof-of-concept for in vivo BCM-imaging was obtained with a Gaussian luciferase linked diabody fusion protein in mouse bioluminescent imaging (BLI). The pancreatic homing and specificity was further confirmed by rat and mice biodistribution studies with radioiodinated IC2, F(ab')<sub>2</sub> and Fab fragments. Recently we have transferred these into formats for NIR noninvasive imaging. Fluorescence-mediated tomography (FMT-NIR) gives the second proof-of-concepts for animal BCM noninvasive imaging using the unique functional beta-cell specificity of IC2.

**Conclusion:** It seems likely to conclude, that IC2 fragments or chimeric engineered formats with an appropriate size potentially could be applied in clinical noninvasive imaging of functional BCM, either with SPECT or PET. Our fifth modality forward in our preclinical trials will be a 4-head NanoSPECT-CT to explore the new chimeric rIC2-hIgG1 and diabody formats further as clinical tracers. The ultimate goal is a full clinical method used for diabetes diagnostic, to follow effects of beta-cell growth promoting drugs, in stem-cell therapy and in islet transplantation.

Supported by: JDRF International and EU-Facilis Programme

### 263

**Longitudinal imaging of transplanted islets in a rat model with SPECT**  
**K.M. Andralojc**, M. Brom, L. Joosten, D.L. Bos, W.J.G. Oyen, O.C. Boerman, M. Gotthardt;  
 Department of Nuclear Medicine, Radboud University Nijmegen Medical Center, Netherlands.

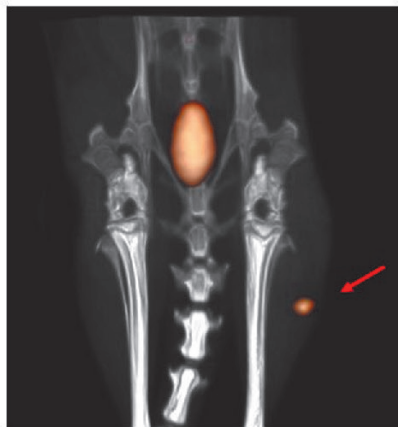
**Aim:** Pancreatic islet transplantation is a promising clinical modality to restore normoglycemia in diabetic patients. However, patients still experience an islet loss immediately after transplantation as well as on the long term. Therefore, there is an urgent need for a method that would non-invasively monitor beta cell mass (BCM) after transplantation *in vivo*. Such a method could give more insight into rejection and could aid to further improve treatment of diabetic patients. We have developed a non-invasive imaging technique that specifically visualizes beta cells *in vivo*. This method is based

on targeting the glucagon-like peptide1 receptor (GLP-1R). The GLP-1R is expressed at high levels on pancreatic beta cells. Our radiolabelled tracer, In-111-labeled Exendin-3, specifically binds to the GLP-1R. We examined whether intramuscularly transplanted islets in rats could be visualized by microSPECT after i.v. injection with radiolabeled Exendin-3.

**Materials & methods:** Islets of Langerhans were isolated from Wag/Rij rats. Various numbers of islets (25–1000) were transplanted into the left thigh muscle of Wag/Rij rats ( $n=20$ ), while vehicle was injected in the right muscle as a control. Rats were monitored up to 14 weeks post-transplantation. SPECT was performed every 1–2 weeks, one hour after i.v. injection of In-111-labeled Exendin-3, using a U-SPECT II microSPECT scanner. After acquiring the last SPECT, the rats were euthanized and the radioactivity in the transplant and other relevant tissues was measured. The muscle with engrafted islets was embedded in paraffin for microautoradiography and immunohistochemistry.

**Results:** The transplanted islets were clearly visualized with SPECT (Fig. 1) at every time-point. Images could be analysed quantitatively. Transplantation of varying numbers of islets revealed an excellent, linear correlation between the SPECT signal and the number of transplanted islets (Pearson  $r=0.99$ ). In rat model, the high sensitivity of the method allowed reliable longitudinal monitoring of the graft during at least 3 months. *Ex vivo* microautoradiography and immunohistochemistry of anti-insulin and anti-GLP-1R staining performed on consecutive histological slices showed high accumulation of In-111-exendin-3 in the beta cells. Anti-insulin staining confirmed that islets after transplantation were viable and produced insulin.

**Conclusion:** In-111-exendin-3 accumulated efficiently in transplanted islets after i.v. injection and targeted insulin producing beta cells via GLP-1 receptor. Transplanted islets could be clearly delineated by microSPECT, allowing long time follow-up. The method is sensitive (25 islets could be visualized) and quantitative as radioactivity in the grafts correlated linearly with the number of transplanted islets. This novel method is reliable, reproducible and robust and holds great potential for non-invasive monitoring of BCM after transplantation in humans.



**Figure 1** 3D volume rendering display of a SPECT/CT of 1,000 islets transplanted in the left hind leg, acquired 1 hour after injection of 15 MBq of In-111-exendin-3 (red arrow indicates 1,000 islets). SPECT/CT was performed 4 weeks post transplantation.

Supported by: FP7/2007–2013 (222980)

## 264

### Quantitative assessment of vascular remodelling in a subcutaneous site following islet transplantation

R. Storrs<sup>1</sup>, R. Krishnan<sup>2</sup>, R. Arora<sup>2</sup>, H. Dogar<sup>2</sup>, M. Lamb<sup>2</sup>, S. White<sup>3</sup>, C. Foster III<sup>2</sup>, E. Botvinick<sup>3</sup>, B. Choi<sup>3</sup>, J.R. Lakey<sup>2</sup>

<sup>1</sup>Islet Sheet Medical LLC, San Francisco, <sup>2</sup>Surgery, University Of California, Irvine, Orange, <sup>3</sup>Beckman Laser Institute and Medical Clinic, University Of California, Irvine, Irvine, USA.

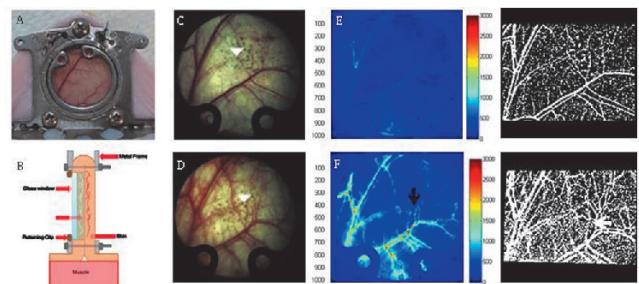
**Background and aims:** Islet transplantation is hampered by a scarcity of human islet donors, inconsistent islet yields and poor function, a problem which may be addressed by xenotransplantation. Islet xenografts require prolonged immunosuppression or biomaterial encapsulation to evade immune-mediated destruction. Although prolonged islet survival with encapsulation has been documented, insufficient oxygen delivery results in suboptimal islet function. Existing models cannot evaluate and quantify the vascular remod-

elling that occurs in response to subcutaneous biomaterial implantation and islet transplantation, non-invasively. We address this problem by employing the mouse dorsal window-chamber model (DWM), Laser Speckle Imaging (LSI) and analyzed changes in subcutaneous functional vascular density (FVD) longitudinally post transplantation.

**Materials and methods:** DWMs were implanted in C57BL/6 albino mice. Host vascular response to introduction of a high guluronate alginate sheet with or without islets (porcine and syngeneic murine) encapsulated within (Fig. 1), was studied over a 7-day period. LSI was performed on days 0 and 7 to map changes in blood flow within. The images obtained were analyzed to evaluate changes in functional vascular density.

**Results:** Vasodilatation and arteriovenous connection formation (Fig. 1D), an increase in the relative rate of blood flow (Fig. 1E) and an increase in the density of functioning capillaries is noted by day 7 (Fig. 1H). The increase in Functional Vascular Density was 45.03%, 43.73% and 65.86% in blank sheets (from  $3.1 \pm 0.11$  to  $4.5 \pm 0.2$ ), sheets containing pig Islets (from  $3.8 \pm 0.15$  to  $5.5 \pm 0.25$ ) and sheets containing mouse Islets (from  $2.7 \pm 0.17$  to  $4.4 \pm 0.13$ ) respectively. The rate of change of Functional Vascular Density is higher in windows containing islet implants ( $1.70 \pm 0.04$ ) as compared to blank sheets ( $1.41$ ).

**Conclusion:** We postulate that all these findings suggest that biomaterial implants can induce angiogenesis which is expected to redound favorably on islet survival. Future experiments will attempt to correlate these findings with islet viability and function as well as extending post transplantation monitoring.



**Fig. 1** DWM Implanted with an alginate sheet containing porcine islets. Lateral view (A). Schematic (B) Bright field Microscopy: Days 0 (C) & 7 (D). LSI performed at 100 ms exposure was used to construct Speckle Flow Index Maps (Day 0) & (Day 7) and Speckle Contrast Maps (not shown). The latter were processed to get Functional Vascular Density Maps (Days 0 (G) & 7 (H)). Black arrows indicate increased blood flow. White arrow heads denotes the islets encapsulated within the sheet.

Supported by: University of California Irvine, Department of Surgery



## PS 001 Autoimmune diabetes

### 265

#### Vitamin D intake during childhood and advanced beta cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes

S.M. Virtanen<sup>1,2</sup>, H.-M. Takkinen<sup>2</sup>, L. Uusitalo<sup>1,2</sup>, S. Niinistö<sup>1</sup>, S. Ahonen<sup>2</sup>, J. Nevalainen<sup>3</sup>, M.G. Kenward<sup>4</sup>, R. Veijola<sup>5</sup>, J. Ilonen<sup>6,7</sup>, O. Simell<sup>8</sup>, M. Knip<sup>9,10</sup>,  
<sup>1</sup>Life Style and Participation, National Institute for Health and Welfare, Helsinki, <sup>2</sup>School of Health Sciences, University of Tampere, <sup>3</sup>Social Research, Statistics, University of Turku, Finland, <sup>4</sup>Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK, <sup>5</sup>Pediatrics, University of Oulu, <sup>6</sup>Virology, University of Eastern Finland, Kuopio, <sup>7</sup>Virology, University of Turku, <sup>8</sup>Pediatrics, University of Turku, <sup>9</sup>the Children's Hospital, University of Helsinki, <sup>10</sup>Pediatrics, University Hospital of Tampere, Finland.

**Background:** Evidence for the role of vitamin D during childhood in the development of beta-cell autoimmunity and clinical diabetes is inconsistent.

**Materials and methods:** Two hundred seventy-four children with repeated positivity for antibodies against islet cells (ICA), together with positivity for at least one of the other three antibodies analyzed or clinical type 1 diabetes (a composite endpoint of advanced beta-cell autoimmunity) were identified from a prospective birth cohort of 7787 infants with HLA-DQB1-conferred susceptibility to type 1 diabetes born in 1996–2004. Three-day food records were completed by the families and day care personnel at 3 to 12 month intervals. Four birth date, gender, area and genetic risk matched controls having been in the follow-up at least until the age when the case became a case were randomly selected for each case.

**Results:** The mean dietary intakes of vitamin D from diet and supplements for 3 and 6 month, 1, 2, 3, 4 and 6 year-olds were 10.4, 12.1, 11.1, 7.9, 5.9, 5.1 and 6.3 µg/day, respectively. Vitamin D intake either from food (OR 1.08, 95% CI 0.97–1.20), supplements (OR 0.96, 95% CI 0.86–1.07) or the total dietary intake (OR 0.96, 95% CI 0.83–1.11) were not associated with advanced beta-cell autoimmunity.

**Conclusion:** In relation to the nutrition recommendations the average intake of vitamin D was suboptimal among these children with HLA-conferred susceptibility to type 1 diabetes. The longitudinal vitamin D intake was not, however, related to the development of advanced beta-cell autoimmunity.

*Clinical Trial Registration Number: NCT00223613*

*Supported by: EFSD/Novo Nordisk grant, Academy of Finland*

### 266

#### Serum levels of soluble receptors for advanced glycation end products decline at seroconversion to autoantibody positivity in prediabetic children

K.M. Salonen<sup>1</sup>, S. Ryhänen<sup>1</sup>, J.M. Forbes<sup>2</sup>, J. Ilonen<sup>3,4</sup>, P.-H. Groop<sup>5,6</sup>, M. Knip<sup>1,5</sup>;

<sup>1</sup>Children's Hospital, University of Helsinki and Helsinki University Hospital, Finland, <sup>2</sup>Baker IDI Heart & Diabetes Institute, Melbourne, Australia, <sup>3</sup>Immunogenetics Laboratory, University of Turku, <sup>4</sup>Department of Clinical Microbiology, University of Eastern Finland, Kuopio, <sup>5</sup>Folkhälsan Research Center, Helsinki, <sup>6</sup>Department of Nephrology, Department of Medicine Helsinki University Central Hospital, Biomedicum Helsinki, Finland.

**Background and aims:** The receptor for advanced glycation end products (RAGE) is a multiligand receptor involved in inflammatory and immune responses. RAGE is suggested to play a role both in the pathogenesis of type 1 diabetes (T1D) and in the development of its complications. The circulating concentrations of soluble RAGE (sRAGE) may be reduced during acute autoimmune processes. This study set out to assess the dynamics of sRAGE during the preclinical T1D disease process.

**Materials and methods:** Serum levels of sRAGE were analyzed in 110 children who progressed to T1D during prospective observation. Samples analyzed were taken at four different time points: (1) before seroconversion to autoantibody positivity; (2) at the time of seroconversion to positivity for one autoantibody; (3) at the time of seroconversion to positivity for multiple ( $\geq 2$ ) autoantibodies; and (4) close to the diagnosis of T1D. Samples of 110 autoantibody-negative controls matched for age, sex, HLA-genotype and place of birth were analyzed for sRAGE at corresponding time points.

**Results:** The progressors had higher levels of sRAGE at all four time points, but the difference was significant only in the first and last sample [mean difference 172 pg/ml,  $p=0.01$  and 164 pg/ml,  $p=0.02$ , respectively (t-test)]. There was a decline in the sRAGE levels in the progressors between the first sample and the second sample [mean sRAGE 1400 pg/ml vs. 1251 pg/ml,  $p=0.001$  (paired t-test)]. A difference in the sRAGE concentrations was also seen when the first sample was compared to the third sample (mean sRAGE 1402 pg/ml vs. 1232 pg/ml,  $p<0.001$ ). There was no such difference in sRAGE levels between the samples taken from the matched controls at corresponding time points. There were no significant differences between the sRAGE levels at seroconversion and the concentrations at T1D diagnosis.

**Conclusion:** Prediabetic children seem to have higher circulating concentrations of sRAGE when compared to controls. A reduction in the circulating sRAGE concentrations coincides with the appearance of diabetes-predictive autoantibodies in children progressing to overt T1D. Whether this reflects a damaging or attempted protective mechanism remains to be defined.

*Supported by: JDFR, Novo Nordisk Foundation and Finnish National CLIGS*

### 267

#### Screening for IA-2 and zinc transporter 8 antibodies identifies relatives of type 1 diabetic patients with a high and age-independent progression rate to clinical onset

E.V. Balti<sup>1</sup>, S. Demeester<sup>1</sup>, A. Van Dalem<sup>1</sup>, O. Costa<sup>1,2</sup>, H. Dorchy<sup>3</sup>, S. Tenoutasse<sup>3</sup>, T. Mouraux<sup>3,4</sup>, C. De Block<sup>5</sup>, P. Gillard<sup>6</sup>, K. Decocchez<sup>1,7</sup>, J.M. Wenzlau<sup>8</sup>, J.C. Hutton<sup>8</sup>, D.G. Pipeleers<sup>1</sup>, I. Weets<sup>1,2</sup>, F.K. Gorus<sup>1,2</sup>;  
<sup>1</sup>Diabetes Research Center, Brussels Free University, <sup>2</sup>Department of Clinical Chemistry and Radio-immunology, University Hospital Brussels, <sup>3</sup>Diabetology Clinic, Queen Fabiola Children's University Hospital, Brussels, <sup>4</sup>Department of Pediatrics, University Hospital Mont-Godinne, Yvoir, <sup>5</sup>Department of Endocrinology, Diabetology and Metabolism, University of Antwerp, <sup>6</sup>Department of Endocrinology, University Hospital Leuven, <sup>7</sup>Department of Diabetology, University Hospital Brussels, Belgium, <sup>8</sup>Barbara Davis Center for Childhood Diabetes, University of Colorado at Denver, Aurora, USA.

**Background and aims:** In first-degree relatives of type 1 diabetic patients, we investigated whether diabetes risk assessment solely based on IA-2 and zinc transporter 8 antibody status (IA-2A, resp. ZnT8A) is as effective as screening for autoantibodies (Abs) against insulin (IAA), GAD (GADA) and IA-2 (with or without ZnT8A) in identifying children, adolescents and adults who rapidly progress to diabetes (within 5 years) in the perspective of constituting homogeneous risk groups for immune interventions before diabetes onset.

**Materials and methods:** Abs were determined by radiobinding assays during follow-up of 6444 siblings and offspring aged 0–39 years at inclusion and consecutively recruited by the Belgian Diabetes Registry.

**Results:** We identified 394 persistently IAA<sup>+</sup>, GADA<sup>+</sup>, IA-2A<sup>+</sup> and/or ZnT8A<sup>+</sup> relatives (6.1%). After a median follow-up time of 52 months, 132 relatives developed type 1 diabetes. In each age category tested (0–9 years, 10–19 years and 20–39 years) progression to diabetes was significantly quicker in the presence of IA-2A and/or ZnT8A than in their joint absence. The 5-year progression rate was age-independent if IA-2A and/or ZnT8A were present at baseline but decreased with age if only GADA and/or IAA were detected ( $P = 0.008$ ). Screening for IA-2A and ZnT8A alone identified 74% of the rapid progressors (vs. 65% if positive for  $\geq 2$  Abs among IAA, GADA and IA-2A) and reduced the group of high-risk Ab<sup>+</sup> relatives to be followed by 63%. Positivity for IA-2A and/or ZnT8A was as sensitive as positivity for at least 2 out of 4 antibodies in individuals aged 10–39 years but less sensitive under age 10, an age group generally not considered for immune interventions until now.

**Conclusion:** Screening for IA-2A and ZnT8A alone allows to identify the majority of rapidly progressing prediabetic siblings and offspring regardless of age and is more cost-effective to select participants for intervention trials than conventional screening.

*Supported by: Belgium Diabetes Reg., JDFR, EU FP7, OZR-VUB, FWO-Flanders, Gepts Fund, NIH*

## 268

**Erythrocyte membrane docosapentaenoic acid levels are associated with islet autoimmunity but not progression to type 1 diabetes. The diabetes autoimmunity study in the young**J.M. Norris<sup>1</sup>, M. Kroehl<sup>1</sup>, T.E. Fingerlin<sup>1</sup>, M. Clare-Salzler<sup>2</sup>, G. Eisenbarth<sup>3</sup>, M. Rewers<sup>3</sup><sup>1</sup>Colorado School of Public Health, Aurora, <sup>2</sup>University of Florida, Gainesville, <sup>3</sup>Barbara Davis Center, Aurora, USA.

**Background and aims:** We previously reported that lower omega-3 fatty acid intake and levels in erythrocyte membranes were associated with increased risk of islet autoimmunity (IA) but not progression to type 1 diabetes (T1D) in children at increased risk for T1D. In the current study, we investigated whether specific fatty acids and genetic markers contributed to this increased risk of IA in the Diabetes Autoimmunity Study in the Young (DAISY).

**Materials and methods:** DAISY is following over 2400 children at increased genetic risk for T1D for the development of persistent IA, as defined as being positive for GAD-65, IA-2, or insulin autoantibodies on two consecutive visits, and then for the development of T1D, as diagnosed by a physician. Using a case-cohort design, erythrocyte membrane fatty acids were measured (as a percent of total lipid) prospectively in 109 children who developed IA, with a mean age at onset of 5.8 years, and in 356 children who remained autoantibody negative. Sixty-five of the IA positive children developed T1D over a mean of 4.7 years of follow-up. Fatty acid levels were analyzed as time-varying covariates using Cox regression.

**Results:** Of the membrane omega-3 fatty acids, docosapentaenoic acid (DPA) was strongly inversely associated (HR: 0.23; 95% CI: 0.09–0.55) with IA risk, while alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) were not, adjusting for HLA-DR status and family history of T1D. None of the membrane omega-6 fatty acids, ie, linoleic acid, gamma linolenic acid, arachidonic acid, were associated with risk of IA. Moreover, none of the omega-3 nor omega-6 fatty acid membrane levels were associated with progression to T1D, adjusting for HLA-DR status, family history of T1D and age at first IA positivity. We cannot examine DPA intake as it is uncommon in the diet. Because membrane DPA levels are determined by endogenous conversion from precursor fatty acids by consecutive desaturation and chain elongation (ALA→EPA→DPA), we hypothesized that omega-3 fatty acid intake would interact with fatty acid desaturation and elongation gene variants to affect risk of IA. We examined 11 variants in *FEN1*, *FADS1*, *FADS2*, *ELOVL2*, *SLC26A10*, *HRH4*, and *SPARC* and total omega-3 fatty acid intake, as determined by annual food frequency questionnaires, on risk of IA. None of the variants were individually associated with risk of IA. However, interactions were detected between omega-3 intake and *FADS1* (rs174556) (interaction  $p=0.037$ ), and *FADS2* (rs174570) (interaction  $p=0.014$ ), adjusting for HLA, family history of T1D, ethnicity, and total energy intake. In children with 1 or 2 *FADS1* minor alleles, increased omega-3 intake is associated with a larger decreased IA risk (HR: 0.60, CI: 0.37–0.97) compared to those without the minor allele (HR: 0.95, CI: 60–1.50), and similarly for children with *FADS2* minor alleles, increased omega-3 intake is associated with a larger decrease in IA risk (HR: 0.42, CI: 0.26–0.69) compared to those with no minor allele (HR: 0.88, CI: 0.60–1.29). Similar interactions were seen for ALA intake alone.

**Conclusion:** The putative protective effect of omega-3 fatty acids on IA may be the result of a complex interaction between intake and genetically-controlled fatty acid desaturation and elongation.

Clinical Trial Registration Number: NIH R01 DK49654, NIH R01 DK32493

## 269

**Analysis of clinical markers for implementation of clinical tool to identify LADA subjects**S. Zampetti<sup>1</sup>, M. Spoleitini<sup>1</sup>, M. Capizzi<sup>1</sup>, G. Campagna<sup>1</sup>, C. Venditti<sup>1</sup>, S. Genovese<sup>2</sup>, A. Giaccari<sup>3</sup>, F. Giorgino<sup>4</sup>, R. Buzzetti<sup>1</sup>, NIRAD Study Group;

<sup>1</sup>Department of Internal Medicine and Medical Specialties, Sapienza, University of Rome, <sup>2</sup>UOC Diabetologia, IRCCS, Sesto San Giovanni, Milano, <sup>3</sup>Department of Endocrinology, University Cattolica, Policlinico Gemelli, Rome, <sup>4</sup>Department of Endocrinology, University of Bari, Italy.

**Background and aims:** Latent autoimmune diabetes of adults (LADA) is characterized by the presence of specific antibodies for autoimmune diabetes, not requiring insulin at diagnosis but having a high risk of progression to insulin dependency. Early diagnosis could have important treatment implications when new therapies to preserve beta-cell function will become avail-

able. The aim of the present study was to develop a clinical screening tool to identify a subpopulation of patients with type 2 diabetes mellitus (T2DM) at high risk of LADA who required GAD antibodies testing.

**Materials and methods:** N=191 LADA subjects and n=382 T2DM patients matched for gender and age, selected from the Non Insulin Requiring Autoimmune Diabetes (NIRAD) study cohort of 4,250 T2DM subjects, were recruited. In all subjects: age at diagnosis, body mass index (BMI), fasting glucose, glycated hemoglobin (HbA1c), waist circumference (WC), total cholesterol, HDL cholesterol, triglycerides and uric acid were evaluated. Statistical analysis was performed using ROC curves (Relative Operating Characteristic) (SPSS v.18). ROC curves allowed us to identify optimal cut-off value indicative for probable presence of GAD antibodies. The cut-off value is that value which corresponds to a point on the ROC curve nearest to the upper left corner of the ROC graph. The area under the ROC curve (AUC) is a scalar measure gauging one facet of performance. Only clinical and biochemical variables whose area under the curve was higher than 0.6 (AUC) were considered for the evaluation of the cut-off.

**Results:** Age at diagnosis, BMI, WC and HbA1c showed an AUC higher than 0.6. These parameters have, therefore, been considered for the evaluation of the cut-off value indicative for the presence of GAD antibodies. The optimal cut-off values were: age of diagnosis <53 years, WC <98.5 cm (males) and <95.5 cm (females), BMI <28.5 kg/m<sup>2</sup> and HbA1c >7.15%. The presence of two or more of these parameters had a 80% sensitivity and 45% specificity with an 0.633 AUC for identifying LADA patients.

**Conclusion:** It is possible to develop a clinical screening tool to increase the probability of identifying LADA patients, however, the analysis of further parameters is essential in order to increase the instrumental clinical prediction specificity.

Supported by: Unconditioned grant from Novo Nordisk

## 270

**Influence of environment, lifestyle, and genes on the risk of LADA (latent autoimmune diabetes in adults) and type 2 diabetes - the ESTRID study**J. Åberg Löfvenborg<sup>1</sup>, M. Dorkhan<sup>2</sup>, L. Groop<sup>2</sup>, B. Rasouli<sup>1</sup>, S. Carlsson<sup>1</sup>;

<sup>1</sup>Institute of Environmental Medicine, Karolinska Institutet, Stockholm,

<sup>2</sup>Department of Clinical Sciences, Lund University, Malmö, Sweden.

**Background and aims:** The prevalence of autoimmune diabetes is, in similarity with type 2 diabetes mellitus (T2DM), increasing worldwide. Latent autoimmune diabetes in adults (LADA) may be the most common form of autoimmune diabetes and yet the risk factors are to a large extent still unknown. With the aim to shed light on common risk factors, the ESTRID study began in 2010. By collecting data on health and lifestyle as well as genetic and clinical information, we aim to identify modifiable environmental and lifestyle factors that could help in preventing autoimmune diabetes.

**Materials and methods:** ESTRID has a case-control design with information collected through questionnaires and blood samples (DNA, anti-GAD, C-peptide). The cases are recruited through the ANDIS and ANDIU (starting 2012) registries, where all incident cases of diabetes in two Swedish provinces (~1 500 000 inhabitants) are registered. LADA cases are distinguished from T2DM cases by measures of anti-GAD. ESTRID recruits all incident cases of LADA, and for each case four T2DM cases and six randomly selected population controls are recruited. The intention is to have gathered information from 800 cases of LADA and 3000 cases of T2DM, together with 3400 controls, by the end of 2014. ESTRID will be the largest population-based study on LADA to date and have enough power to detect even moderately increased risks. With this data we aim at studying the risk of LADA/T2DM in relation to diet, alcohol, tobacco, physical activity, sleep, infections, psychosocial and socioeconomic factors. For the cases, questionnaire data is collected in close proximity to diagnosis since the information should reflect lifestyle factors that may have affected diabetes onset. Genetic information of > 130 gene variants is available from ANDIS and ANDIU. Controls for the genetic analyses will be obtained from the ongoing epidemiological studies EIRA (rheumatoid arthritis) and EIMS (multiple sclerosis). By using cases from ESTRID and controls from EIRA/EIMS, we will study gene-environment interaction in relation to LADA and T2DM.

**Results:** Since the start in 2010, 672 cases of diabetes (159 LADA and 513 T2DM) together with 768 healthy controls have been recruited, with high response rate in both cases (80.1%) and controls (69.9%). Mean BMI (kg/m<sup>2</sup>) did not differ between LADA cases and controls (27.7 vs. 26.1) but was higher in T2DM (30.6),  $p<0.0001$ . We also find that both LADA and T2DM were more common in men (57.7% and 59.9% of cases were men, as compared to 47.9% of controls). Thus, the risk is markedly lower for women (OR

for LADA=0.58, 95% CI=0.35–0.96). With regard to risk factors, preliminary findings based on a very limited number of cases and a crude assessment of diet indicate that consumption of whole grain bread may reduce the risk of LADA (OR=0.77, 95% CI=0.40–1.50) as well as T2DM (OR=0.57, 95% CI=0.3–1.09). Similar findings are seen for LADA regarding daily consumption of muesli (OR=0.60, 95% CI=0.23–1.56).

**Conclusion:** Being the largest study of its kind, ESTRID provides unique opportunities to study the etiology of LADA. We aim to identify environmental and lifestyle factors affecting disease onset, as well as to contribute to the currently limited knowledge of gene-environment interaction in relation to LADA and T2DM, with the overall goal to find ways to prevent autoimmune diabetes.

Supported by: Swedish Research Council, AFA Insurance, Swedish Diabetes Association

## 271

### Study of phenotypic characteristics of patients with latent autoimmune diabetes in adults (LADA) in a Danish population

M. Wod<sup>1</sup>, A. Brandstorp-Boesen<sup>2</sup>, J.Ø. Rathe<sup>3</sup>, K.B. Yderstræde<sup>1</sup>, H. Beck-Nielsen<sup>1</sup>, K. Højlund<sup>1</sup>

<sup>1</sup>Department of Endocrinology, Odense University Hospital, Denmark,

<sup>2</sup>Dermatology, Oslo Universitets sykehus, Rikshospitalet, Oslo, Norway,

<sup>3</sup>Research Unit of General Practice, University of Southern Denmark, Odense, Denmark.

**Background and aims:** Latent autoimmune diabetes in adults (LADA) is the second most common form of diabetes and is considered less understood than its much better characterized counterparts of type 1 diabetes (T1D) and type 2 diabetes (T2D). There is still little agreement on the diagnostic criteria for LADA and therefore it is important to characterize this large subgroup of diabetic patients. A database of newly referred diabetic patients to Odense University Hospital, Denmark tested for anti-GAD65 positivity between 1997 and 2011 is in the process of being established with the aim to investigate phenotypic differences between patients with LADA, T1D and T2D, respectively. This study reports preliminary data from the period 1997–2001.

**Materials and methods:** Anti-GAD65 titer was positive in 226 of 1332 diabetic patients tested between 1997 and 2001. This study includes 54 anti-GAD65 positive diabetic patients, for whom 1) diabetes duration was less than 5 years, and relevant anthropometric and biochemical data were readably available. Thirty-three patients with age at diagnosis > 35 years were defined as LADA, and the others as T1D (n=19). A random group of anti-GAD65 negative diabetic patients with age at diagnosis > 35 years, for whom data were available, was selected among the 1056 anti-GAD65 negative patients, and defined as T2D (n=44). Data included age, gender, BMI, blood pressure, anti-GAD65 levels, HbA1C, and fasting levels of glucose, C-peptide, and lipid profile and are given as mean ± SD.

**Results:** No differences in age (50.9 ± 10.4 vs. 50.8 ± 7.3 years), BMI (27.3 ± 5.5 vs. 29.8 ± 7.9 kg/m<sup>2</sup>), HbA1c, blood pressure, fasting glucose or cholesterol levels were seen between patients with LADA and T2D. Patients with LADA had lower levels of fasting C-peptide (608 ± 403 vs. 918 ± 593 pmol/l; p<0.01) and plasma triglyceride (1.94 ± 1.50 vs. 2.92 ± 2.16 mmol/l; p<0.05) than T2D patients. Compared with the younger (26.6 ± 5.4 years) T1D patients, BMI (27.3 ± 5.5 vs. 23.9 ± 4.43 kg/m<sup>2</sup>; p<0.05) and fasting C-peptide I (608.1 ± 403.4 vs. 280.5 ± 224.5 pmol/l; p<0.01) were higher in LADA patients. Moreover, anti-GAD65 titers were almost 2-fold lower (p<0.01) in LADA than in T1D patients.

**Conclusion:** These preliminary results indicate that LADA patients differ from age-matched type 2 diabetic patients in terms of lower fasting C-peptide and triglyceride levels, and from younger T1D patients by higher BMI, higher fasting C-peptide levels and lower anti-GAD65 titers. Further establishment of a database for the remaining anti-GAD65 positive diabetic patients in the period 1997–2001 and the expected approximately 450 anti-GAD65 positive diabetic patients in the period 2002–2011 will offer the opportunity to search for clinical diagnostics tools to improve the discrimination between patients with LADA, T1D and T2D.

## 272

### Hyperbolic product, cardiometabolic profile and thyroid autoimmunity in phenotypic type 2 diabetics with GAD65 antibodies

Y. Mahadeb, D. Gruson, M. Buysschaert, M.P. Hermans; Endocrinology, Saint-Luc University Clinics, Brussels, Belgium.

**Introduction:** Type 2 diabetes (T2DM) usually results from a combination of insulin resistance and β-cell deficiency, while T1DM results from β-cell destruction associated with islet autoantibodies, including those directed against glutamate decarboxylase (GAD<sub>65</sub> antibodies [GADA]). The clinical impact of low GADA positivity (<10 U/ml) in T2D is debated, especially since screening is rarely performed in routine clinical care and, in addition, there exists a slight subclinical GADA positivity titer in the general population.

**Aims:** To determine the prevalence and the cardiometabolic/autoimmune phenotype of GADA[+] in 524 T2DM patients. Glucose homeostasis determinants were assessed by HOMA: insulin sensitivity (HOMA S); β-cell function (HOMA B); the hyperbolic product [BXS]; and the annualized loss in [BXS].

**Results:** GADA prevalence was 6% (n=30). GADA[+] patients were compared to n=494 GADA[-] controls. There were no differences between groups for age, diabetes duration, and family history of diabetes. There was a higher proportion of women (33 vs. 53%) in GADA[+]. There were no differences between groups in BMI, waist circumference, body fat or visceral fat. HOMA S (mean±1 SD) was lower than normal, with no difference between groups. HOMA B was 52 (38)% (GADA[+]) vs. 61 (43)% (GADA[-]; NS) and the hyperbolic product [BXS] was 29 (19)% (GADA[+]) vs. 27 (17)% (GADA[-]; NS). The annualized rate of [BXS] loss was 1.26 (0.46)%/year (GADA[+]) vs. 1.34 (0.51)%/year (GADA[-]; NS). A metabolic syndrome was present in 83% (GADA[+]) vs. 80% (GADA[-]; NS). There were no differences between groups for classes of oral antidiabetic agents or intensity of glucose-lowering therapy: an OAD mono-, bi- and tri-therapy (± insulin) was prescribed to 20; 10; and 0% (GADA[+]) vs. 17; 24; and 2% (GADA[-]; NS). Insulin, combined with OAD or as monotherapy was prescribed to 36 and 27% of GADA[+] vs 32 and 17% of GADA[-] (NS). Duration of diabetes before insulin therapy was 9 (9) (GADA[+]) vs. 9 (7) years (GADA[-], NS). HbA<sub>1c</sub> was 7.8 (1.5)% (GADA[+]) vs. 7.6 (1.5)% (GADA[-]; NS). In the whole group, prevalence of autoimmune thyroid disease (AITD, anti-TG and/or TPO) was 10%. In GADA[+], this prevalence was significantly increased and equally affected the two sexes: 29% (men), and 25% (women), while for GADA[-] the prevalence was 5% (men) vs. 18% (women; p<0.0001). Micro- and macroangiopathic complications did not differ between groups.

**Conclusion:** The presence of low-titer GADA autoimmunity among unselected T2DM patients was not associated with accelerated β-cell function, nor with any distinctive cardiometabolic phenotype, but for a markedly increased prevalence of autoimmune thyroid disease, especially among men.

## 273

### Zinc transporter type 8 (ZnT8) autoantibodies: prevalence and phenotypic associations in diabetic patients diagnosed >35 years

M.K. Andersen<sup>1,2</sup>, T. Härkönen<sup>3</sup>, P.-H. Groop<sup>2,4</sup>, M. Knip<sup>2,3</sup>, T. Tuomi<sup>1,2</sup>,

<sup>1</sup>Department of Medicine, University of Helsinki, <sup>2</sup>Folkhälsan Research Center, Helsinki, <sup>3</sup>Children's Hospital, University of Helsinki and Helsinki University Central Hospital, <sup>4</sup>Division of Nephrology, Helsinki University Central Hospital, Finland.

**Background and aims:** ZnT8 is an antigen in type 1 diabetes. The ZnT8 protein is encoded by *SLC30A8* where the C-allele of the R325W variant is associated with type 2 diabetes and reduced beta-cell function in non-diabetic subjects. The aim of this study was to assess the prevalence of ZnT8 autoantibodies (ZnT8A) in adult-onset diabetic patients, and to characterize associations between ZnT8A and phenotype, as well as *SLC30A8* and *HLA-DQB1* genotype.

**Materials and methods:** The R and the W isoforms of the ZnT8A were measured in Finnish diabetic patients diagnosed after the age of 35 years (type 1 diabetes: n=274; Latent autoimmune diabetes in adults (LADA): n=273). All patients and 537 non-diabetic control subjects were genotyped for the R325W variant in *SLC30A8* (rs13266634) as well as risk and protective alleles in the *HLA-DQB1* region.

**Results:** ZnT8A were significantly more prevalent in LADA (31.0%) compared with adult-onset type 1 diabetic patients (18.7%, p=0.001). Among the LADA patients, ZnT8A were associated with higher age [ZnT8A posi-



tive vs. ZnT8A negative: 67.4 (19.4) vs. 61.3 (13.0) years,  $p=0.001$ ], higher age at diagnosis [60.0 (17.0) vs. 52.0 (15.3) years,  $p=0.001$ ], and lower insulin secretion during OGTT [incremental AUC S-C-peptide: 1.92 (1.66) vs. 3.60 (2.73) nmol/l,  $p=0.021$ ], whereas no association was observed with duration. Among the adult-onset type 1 diabetic patients, ZnT8A were associated with lower age [47.2 (14.3) vs. 52.9 (11.9) years,  $p=0.003$ ], and shorter duration [4.4 (6.0) vs. 10.8 (11.2) years,  $p<0.0001$ ]. The CC genotype of the R325W variant was associated with LADA with GADA levels below the median [*SLC30A8* CC: OR (95% CI): 1.55 (1.06 - 2.27),  $p=0.024$ ]. No association was observed between the variant and LADA with GADA levels above the median or adult-onset type 1 diabetes. Among the non-diabetic subjects and the LADA patients the C-allele of the R325W variant was associated with reduced insulin secretion [incremental S-C-peptide: *SLC30A8* CC/CT vs. TT: 2.37 (2.40) vs. 3.67 (3.47) nmol/l,  $p=0.007$ ]. ZnT8A prevalence was non-significantly higher in carriers of protective (40.5%) compared to neutral (27.8%) and risk (31.9%) associated *HLA-DQB1* genotypes.

**Conclusion:** The prevalence of ZnT8A was higher, and the autoantibodies did not disappear with longer duration to the same degree in LADA compared with adult-onset type 1 diabetic patients. The *SLC30A8* CC genotype was associated with LADA with low GADA levels. In LADA patients in general, both *SLC30A8* genotype and the presence of ZnT8A affected insulin secretion.

## PS 002 Genetics of type 1 diabetes

### 274

#### Association analysis of 31 type 1 diabetes susceptibility loci in Finnish families

A.P. Laine<sup>1</sup>, J. Ilonen<sup>1,2</sup>, M. Knip<sup>3,4</sup>, The Finnish Paediatric Diabetes Register; <sup>1</sup>University of Turku, <sup>2</sup>University of Eastern Finland, Kuopio, <sup>3</sup>University of Helsinki and Helsinki University Central Hospital, <sup>4</sup>Folkhälsan Research Center, Helsinki, Finland.

**Background and aims:** In addition to the HLA-region conferring major genetic determinants of risk and protection, more than 50 confirmed non-HLA T1D susceptibility loci have been identified in candidate gene studies or in genome wide association studies. The association evidence for many of these loci is based solely on findings in large admixed European and/or North American populations. To investigate the role of these loci in a less heterogeneous population, we have analyzed 36 SNP markers in 31 independent loci for association with T1D in 1761 T1D trio families from Finland. 25 of these loci are considered confirmed, while the evidence for the remaining six loci is sporadic. To explore the possible heterogeneity of effect, the data set was split up based on the affected child's HLA-haplotypes, age at diagnosis and sex.

**Materials and methods:** All subjects with T1D were diagnosed under the age of 15 years according to the WHO criteria. SNP markers were genotyped using the Sequenom platform. The CCR5 delta 32 deletion (rs333) was genotyped using fluorescently labelled PCR primers and automated sequencer. All statistical testing was carried out in the PLINK v1.07 software. Transmission/disequilibrium test (TDT) was used for testing association. TDT p-values were adjusted for multiple testing using a false discovery rate step-up procedure. We consider p-values  $<0.05$  significant for the confirmed loci and adjusted p-values  $<0.05$  for the non-confirmed loci. Breslow-Day (BD) test was used for testing heterogeneity of odds ratio (OR) between subgroups.

**Results:** 17 of the 31 loci showed significant association in our data set. The strongest effect was seen with INS, PTPN22 and IL2RA (respective ORs of 0.44, 1.79 and 0.61), while the rest of the loci ranged from OR=1.31 to no effect. We did not observe any conspicuous differences in effect size compared to the latest meta-analyses, and the direction of effect for each allele was consistent with prior findings. 14 loci did not show association in our study. Five of them were non-confirmed loci and six lacked power to detect their respective discovery effects. Three confirmed loci with good power (assuming discovery effect and our observed allele frequency) were not detected. One of them, CD226, still showed significant association in the 0-4 years subgroup based on age at diagnosis, but CYP27B1 and PRKCQ failed to show any T1D association in the current data set. Significant OR heterogeneity was seen with PTPN22, CTSB and FUT2 (HLA, respective BD p-values of 0.0069, 0.031 and 0.0037) and with LOC150577, INS and CD226 (age at diagnosis, respective BD p-values of 0.046, 0.039 and 0.022) and with LOC646538 and CD69 (sex, respective BD p-values of 0.0090 and 0.035).

**Conclusion:** Our association findings in the Finnish population corresponded well to those reported in the latest meta-analyses using large admixed European and/or North American populations. Lack of population specific susceptibility is not surprising since the confirmed T1D loci have been discovered and replicated mostly in diverse populations and are therefore likely to be universal. The observed significant effect modifications by subgrouping could be an indication of underlying complexity of T1D susceptibility although they need independent confirmation before any claims can be made about their importance.

### 275

#### Genetic risk score predicts beta cell function in children with new onset type 1 diabetes

L. Nielsen<sup>1</sup>, M.M. Andersen<sup>1</sup>, S. Poerksen<sup>1</sup>, C. Brorsson<sup>2</sup>, S. Fredheim<sup>1</sup>, L. Hansen<sup>1</sup>, F. Pociot<sup>2</sup>, H.B. Mortensen<sup>1</sup>;

<sup>1</sup>Department of Pediatrics, Herlev Hospital & University of Copenhagen, Herlev, <sup>2</sup>Glostrup Research Institute, Glostrup Hospital & University of Copenhagen, Glostrup, Denmark.

**Background and aims:** The natural course of type 1 diabetes in newly diagnosed children is characterized by a substantial diversity between individuals. Some children experience a relatively mild onset with limited need of exogenous insulin, while others are more aggressively attacked and require intensive insulin treatment from onset. This variation in disease severity is

poorly understood. The aim of this study is to investigate if the inter-individual variation in disease manifestation can be explained by the patient's genetic background. Therefore, we have investigated the combined impact of several risk alleles of SNPs in type 1 or type 2 diabetes susceptibility genes on the residual beta-cell function and glycaemic control during disease progression in children with new onset type 1 diabetes.

**Materials and methods:** The study is a multicenter longitudinal investigation with 18 participating paediatric centres from 15 countries in Europe and Japan (84% Caucasians). 275 children and adolescents less than 16 yr were included in the study. The residual beta-cell function was estimated by a meal-stimulated C-peptide test 1, 6 and 12 months and capillary samples for centrally determined HbA<sub>1c</sub> were collected 1, 3, 6, 9 and 12 months after disease onset at each center and insulin regimens were recorded. Insulin dose-adjusted HbA<sub>1c</sub> (IDAA1c) was calculated as HbA<sub>1c</sub> % + (4 x insulin dose (units per kilogram per 24 h)). Genotyping of common SNPs in the following genes: *INS*, *TNFAIP3*, *ERBB3*, *IL2RA*, *WFS1*, *HHEX-IDE*, *PPARG* and *SLC30A8* were done by KBioscience using an in-house KASPar assay. The criterion for the SNPs to be included into the risk score model was an individual genotype effect ( $p < 0.05$ ) on stimulated C-peptide during disease progression. The eight qualified SNPs were analyzed for association with disease progression. Main outcome measures for disease progression were described by glycaemic control as assessed by HbA<sub>1c</sub>/ IDAA1c or residual beta-cell function as assessed by stimulated C-peptide.

**Results:** In a multiple regression analyses adjusting for age, sex and HLA risk groups we found a highly significant negative association between numbers of risk alleles and stimulated C-peptide at 1, 6 and 12 months after onset ( $p < 0.0001$ ). In line with this we found a positive significant association between numbers of risk alleles and IDAA1c from 3 until 12 months after onset ( $p = 0.0003, 0.004, 0.007$  and  $0.003$  for 3, 6, 9 and 12 months visits, respectively) when adjusted for age, sex and HLA risk groups. This effect was not seen for the individual SNPs.

**Conclusion:** This study demonstrates large differences in the residual beta-cell function and glycaemic control during disease progression depending on individual genetic background. Thus, it seems we might have different diabetic phenotypes suggesting diabetes management should be individualized already at disease onset where some children may need an insulin pump or other intensified treatment regimens. Furthermore, a combined genetic risk score instead of individual SNPs might improve the association to clinical endpoints and could be considered an important tool when evaluating the pharmacogenetic effect after intervention studies.

## 276

### Baseline patterns and genetic fingerprints related to residual beta cell function and ZnT8 autoantibody profiles in Danish children with new onset type 1 diabetes

S. Pörksen<sup>1</sup>, M.L.M. Andersen<sup>1</sup>, M.A. Rasmussen<sup>2</sup>, J. Svensson<sup>1</sup>, N.T. Hertel<sup>3</sup>, F. Pociot<sup>4</sup>, J.S. Petersen<sup>5</sup>, L. Hansen<sup>1</sup>, H.B. Mortensen<sup>1</sup>, L.B. Nielsen<sup>1</sup>; <sup>1</sup>Pediatrics, Herlev University Hospital, <sup>2</sup>Food Science, Quality and Technology, University of Copenhagen, <sup>3</sup>Pediatrics, Odense University Hospital, <sup>4</sup>Biochemistry, Diagnostic Unit, Glostrup University Hospital, <sup>5</sup>Diabetes Biology & Pharmacology, Novo Nordisk A/S, Måløv, Denmark.

**Background and aims:** The natural history of type 1 diabetes (T1D) disease progression is complex. The purpose of the present remission phase study in a Danish cohort of children with new onset T1D is to explore the natural history of T1D the first 12 months after diagnosis by use of latent factor models which describe unique dynamic profiles of clinical biomarkers related to baseline characteristics and genetic profiles of T1D and T2D related SNPs.

**Material and methods:** We followed 129 children aged < 17 years 12 months after onset of T1D. The data are organized as three individual data blocks: A) Characteristics registered at onset (sex, age, duration of symptoms, HbA<sub>1c</sub>, blood glucose, std. bicarbonate and HLA risk groups); B) Clinical and para-clinical markers (stimulated C-peptide and proinsulin, autoantibodies, HbA<sub>1c</sub>, stimulated blood glucose, daily insulin dose and BMI) collected 1, 3, 6, 9 and 12 months after diagnosis and C) Genotyping of 52 SNPs associated with T1D or T2D in GWA studies. The aim of the statistical analysis is to extract and relate biologically intuitive patterns common between the different data blocks using a multiblock procedure (Coupled Matrix Tensor Factorization). To confirm the validity of the final model, the same patterns of clinical and laboratory characteristics were investigated in the international Hvidoere Remission Phase cohort - an independent, similar cohort of newly diagnosed children.

**Results:** The model with lowest residual variation was chosen and by use of this model two components of relevance, explaining 21.6% of the total variation in the dataset, was identified. The first component assigned 'beta-cell function', described a decrease in stimulated C-peptide and proinsulin levels in combination with an increase in stimulated blood glucose, glucagon and daily insulin dose per kg bodyweight during the first 12 months after onset. Furthermore this component was positively associated with the number of risk alleles of *WFS1*, *CDKN2A/2B* and *RNLS* ( $p = 0.0004$ ), and in addition positively associated with baseline characteristics such as young age, presence of DKA and long duration of disease symptoms ( $p = 0.006$ ). The second component assigned 'ZnT8 autoantibodies (ZnT8Ab)' indicated that high ZnT8Ab, which is related to better residual beta-cell function, and low postprandial glucagon levels are associated with a combined genetic profile of risk alleles from SNPs in type 1 diabetes related genes: *IFIH1*, *TCF2*, *TAF5L*, *IL2RA* and *PTPN22* and protective alleles from the *ERBB3* gene ( $p = 0.0005$ ). The derived patterns where found replicable in the independent Hvidoere Remission Phase Cohort.

**Conclusion:** In conclusion, complex latent factor analysis on data from a clinically well characterised Danish Remission Phase Cohort can describe patterns present at diagnosis which is associated to residual beta cell function and glycaemic control during the first 12 months of clinical disease and identify humoral immune responses and genetic interacting factors that were not recognised by classical univariate analyses

## 277

### Polymorphisms in the INS and IKZF4 genes are associated with insulin autoantibodies at the diagnosis of type 1 diabetes

J. Lempainen<sup>1</sup>, A.P. Laine<sup>1</sup>, M. Knip<sup>2,3</sup>, J. Ilonen<sup>1,4</sup>, The Finnish Paediatric Diabetes Register;

<sup>1</sup>University of Turku, <sup>2</sup>University of Helsinki and Helsinki University Central Hospital, <sup>3</sup>Folkhälsan Research Center, Helsinki, <sup>4</sup>University of Eastern Finland, Kuopio, Finland.

**Background and aims:** More than 50 loci outside the HLA region have been confirmed to affect type 1 diabetes (T1D) risk. However, their effect on the beta-cell autoimmunity is poorly described. We analysed the association of 35 SNP markers from 31 independent loci previously associated with T1D with the presence of T1D-associated autoantibodies at the time of T1D diagnosis.

**Materials and methods:** The study cohort comprised 1556 children from the Finnish Paediatric Diabetes Register. All subjects were diagnosed with T1D before 15 years of age between years 1998 and 2009. Serum samples were collected within 14 days after the T1D diagnosis (mean 5.2 days). SNPs were genotyped using the Sequenom platform. The association between various genotypes and positivity for antibodies against islet cells, insulin (IAA), glutamic acid decarboxylase, islet antigen 2 and zinc transporter-8 (ZnT8A) were analysed using Chi Square test and corrected for multiple testing (31 loci).

**Results:** INS gene polymorphism rs689 was strongly associated with IAA positivity at the time of T1D diagnosis ( $p = 0.00012$ ) but not with positivity to other autoantibodies tested. In addition, the IKZF4 C/A polymorphism (rs1701704) and rs2292239 in the nearby ERBB3 gene showed association with IAA ( $p = 0.014$  and  $0.031$ , respectively) but were not associated with other autoantibodies. rs6546909 in the DQX1 gene showed borderline association with ZnT8A ( $p = 0.031$ ). The other SNPs analysed showed no significant association with any autoantibodies tested. The presence of T1D risk associated INS AA genotype was associated with IAA, 544 (44.8%) subjects with AA genotype were IAA positive compared to 89 (29.4%) and 7 (30.4%) with AT and TT genotypes, respectively. In contrast, the presence of the T1D-risk associated C allele of the IKZF4 marker was inversely associated with IAA. 66 (34.0%) subjects homozygous for the disease associated C allele and 286 (38.5%) subjects with the heterozygous CA genotype were IAA positive compared to 278 (47.4%) with the AA genotype.

**Conclusion:** While the INS gene risk genotype was associated with the presence of IAA, the IKZF4 T1D risk allele was inversely associated with IAA. In the same cohort, the OR for the T1D risk effect of the C allele was 1.3, and its effect was strongest among subjects diagnosed between 10 to 15 years of age. These results suggest that the IKZF4 risk allele is more important among subjects with beta-cell autoimmunity initiated later during childhood. The IKZF4 gene encodes a zinc finger protein specifically expressed in lymphocytes and is implicated in the control of lymphoid development. Our results suggest that the IKZF4 polymorphism is included in a pathway of beta-cell autoimmunity alternative for the route characterized with IAA and development of T1D during early childhood.

## 278

# Immunogenetic determinants of childhood type 1 diabetes in European and non-European immigrants to Sweden in the Better Diabetes Diagnosis Study

A.J. Delli<sup>1</sup>, B. Lindblad<sup>2</sup>, A. Carlsson<sup>3</sup>, H. Elding-Larsson<sup>1</sup>, G. Forsander<sup>2</sup>, S.A. Ivarsson<sup>1</sup>, J. Ludvigsson<sup>4</sup>, I. Kockum<sup>5</sup>, C. Marcus<sup>6</sup>, U. Samuelsson<sup>7</sup>, E. Örtqvist<sup>8</sup>, L. Groop<sup>1</sup>, Å. Lernmark<sup>1</sup>;

<sup>1</sup>Department of Clinical Sciences, Lund University, Malmö, <sup>2</sup>Department of Pediatrics, Sahlgrenska Academy, University of Gothenburg, <sup>3</sup>Department of Pediatrics, Lund University, Lund, <sup>4</sup>Department of Clinical and Experimental Medicine, Linköping University, <sup>5</sup>Department of Clinical Neurosciences, Karolinska Institute, Stockholm, <sup>6</sup>Department of Clinical Science, Intervention and Technology, Karolinska Institute, Stockholm, <sup>7</sup>Diabetes Research Center, Linköping University Hospital, <sup>8</sup>Department of Woman and Child Health, Karolinska Institute, Stockholm, Sweden.

**Background and aims:** We examined whether non-Europeans compared to European and Swedish patients, present different genetic determinants associated with their T1D and whether these determinants affected the types and frequencies of islet autoantibodies and metabolic indicators at diagnosis.

**Materials and methods:** We recruited 2538 newly diagnosed T1D patients (<18y, 55% male) from the Better Diabetes Diagnosis (BDD) study. Country of birth of all parents and grandparents identified three subgroups; non-Europeans (148; 5.8%) and Europeans (86; 3.5%) and Swedes (2304; 90.7%)

**Results:** The HLA-DQ2/x (where x is any other haplotype except DQ8) predominated the non-Europeans (46%), compared to Europeans (26%;  $p=0.002$ ) and Swedes (15%;  $p=0.0005$ ). Furthermore, the none-DQ2-non-DQ8 (x/x) was more prevalent in non-Europeans (26%) compared to Europeans (23%;  $p=0.68$ ) and Swedes (14%;  $p=0.0005$ ) who had more DQ8/x (41%;  $p=0.0005$ ) and DQ2/8 (31%;  $p=0.0005$ ), compared to non-Europeans and Europeans. The PTPN22 (R620W) risk T allele (CT+TT) was extremely uncommon (5%) in the non-Europeans, compared to 14% in Europeans and 35% in Swedes ( $p=0.0005$ ). The T2D-associated zinc transporter 8 gene (SLC30A8) CC genotype dominated the non-Europeans (72%), compared to 50% in Europeans and 46% in Swedes ( $p=0.0005$ ). The FTO AA risk genotype was slightly more frequent in non-Europeans (24%) than Europeans (14%;  $p=0.12$ ) and Swedes (17%;  $p=0.051$ ). Overall, the FTO AA genotype was negatively associated with multiple autoantibodies ( $p=0.003$ ) and ZnT8-Tryptophan (W) antibodies ( $p=0.015$ ). The frequencies and types of islet autoantibodies were closely related to the genetic determinants. Consequently, non-Europeans were more likely to be autoantibody negative than Europeans and Swedes (11%, 8% and 6% respectively;  $p=0.07$ ) and less likely to develop multiple ( $\geq 2/6$ ) autoantibodies (64%, 81% and 79% respectively;  $p=0.005$ ). In the immigrant group (non-Europeans and Europeans), higher levels of mean HbA1c (mmol/mol) (105; SD=26 and 108; SD=25 respectively) than Swedes (94; SD=27) were observed in females ( $p=0.0005$ ) but not males ( $p=0.85$ ). Although the prevalence of DKA at diagnosis was less in Swedes (15%) than non-Europeans (22%) and Europeans (23%),  $p=0.009$ ; Swedish patients who develop DKA were older ( $p=0.0005$ ).

**Conclusion:** Our findings showed a gradient-like pattern in presence of immunogenetic markers of T1D. Non-Europeans, compared to Europeans and Swedes, develop diabetes with less T1D risk genes and more T2D-associated genes and therefore they had lesser islet autoantibodies suggesting a possibility of shared T1D and T2D pathways in their susceptibility to develop diabetes.

Supported by: Barndiabetesfonden

## 279

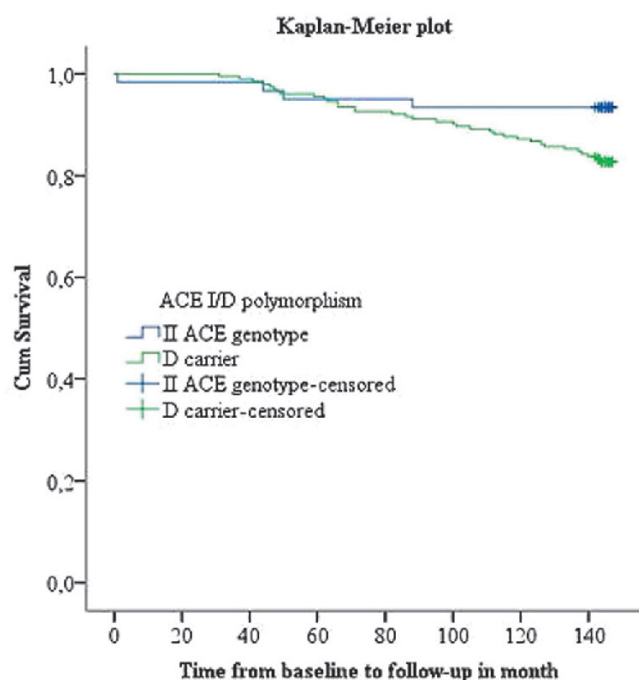
# ACE genotype and phenotype and 12-year all-cause mortality in type 1 diabetes

L.H. Færch<sup>1</sup>, A.-S. Sejlind<sup>1</sup>, U. Pedersen-Bjergaard<sup>1</sup>, B. Thorsteinnsson<sup>1,2</sup>;

<sup>1</sup>Dept. of Cardiology Nephrology and Endocrinology 0652, Hypoglycaemia research group, Hillerød, <sup>2</sup>Faculty of Health, Copenhagen, Denmark.

**Background and aims:** The D allele of the ACE I/D polymorphism, which confers the phenotype of high ACE activity, is implicated in increased morbidity in type 1 diabetes. D-allele carriage is associated with faster progression of diabetic nephropathy, development of cardiac hypertrophy, and hypertension. Diabetic nephropathy is associated with increased mortality. In both type 1 diabetes and in general cardiac hypertrophy and hypertension are associated with increased mortality. We studied whether D-allele carriage and high serum ACE activity are as-

sociated with overall mortality after 12 years in a type 1 diabetic cohort. **Materials and methods:** A cohort of 265 adult patients with type 1 diabetes was recruited in 1999 to 2000 and classified according to ACE I/D polymorphism, serum ACE activity and treatment with RAS blocking agents. After 12 years the cohort was followed up for all-cause mortality. All patients were included in the genotype analysis, whereas only subjects not treated with any RAS blocking agents ( $n=166$ ) - and thus with un-modified spontaneous ACE activity - were included in the phenotype analysis. The cohort consisted of 157 men, with a median diabetes duration of 20 years. Nephropathy was present in 25 percent and 20 percent of the cohort were on ACE inhibitors. We used Kaplan-Meier method to evaluate the mortality rate for both genotype and ACE activity.  $P<0.05$  (two-sided) was considered to be significant. **Results:** A total of 39 patients (15 %) died during follow-up. Mortality rate was lower in II-carriers compared to the D-carriers, 6.6 % vs. 17.2 % ( $p=0.048$ ). This is depicted in the Kaplan-Meier plot below. Thirteen of the patients not on RAS blocking treatment (7.8 %) died during follow-up. The mortality rate of zero in the lowest ACE quartile was lower than that of the three upper ACE quartiles 10.6% ( $p=0.029$ ). **Conclusion:** In type 1 diabetes carriage of the D-allele of the ACE I/D polymorphism - and correspondingly high spontaneous serum ACE activity - is associated with increased long-term all-cause mortality in a Kaplan-Meier survival plot.



Supported by: EFSD/JDRF/Novo Nordisk grant

## 280

# The promoters of genes associated with type 1 and type 2 diabetes seem to have some specific features

C. Ionescu-Tirgoviste<sup>1</sup>, P. Gagniuc<sup>2</sup>, C. Guja<sup>1</sup>;

<sup>1</sup>1st Clinic of Diabetes, National Institute of Diabetes, Nutrition and Metabolic Diseases, <sup>2</sup>Institute of Genetics, Bucharest, Romania.

**Background and aims:** Genome-wide association studies (GWAS) made in the last decade raised the number of genes associated with type 1 (T1D) and type 2 (T2D) diabetes to about 50 genes for each. The environmental factors seem to play an important role in the expression of these genes through transcription factors that act on promoters. For this reason in this paper we tried to analyze by using an original method the promoter sequences (about 500 pb) of various genes associated with the two main phenotypes of diabetes, which may shed a new light on the diabetogenic mechanisms.

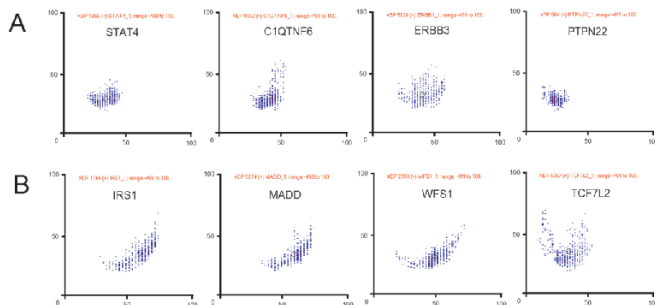
**Materials and methods:** Using available databases we analyzed a total of 25 promoters of genes associated with T1D and T2D. In order to measure the structural features of promoters sequences, we used our original method of analysis based on Kappa Index of Coincidence (Kappa IC). Kappa IC is a cryptographic method not implemented so far in DNA sequence analysis.



Our method measures the degree of randomization of a DNA sequence. Unlike sequence alignment algorithms, our method uses a comparison between the frequency and the nucleotide content of a promoter sequence. Among the analyzed promoters of genes associated with T1D, we mention the C1QTNF6, CD55, ERBB3, PTPN22 and STAT4 gene promoters (Figure 1A) and for T2D the CAMK1D, CDC123, CDKAL1, CDKN2AIP, DUSP9, HHEX, HMG2A, HNF1B, IRS1, NOTCH2, PPARG, PROX1, TCF7L2, THADA, TLE4, TP53INP1, TSPAN8, VPS13C, WFS1 and ZFAND6 gene promoters (Figure 1B).

**Results:** Our analysis shows that the promoters associated with T1D contain similar patterns that may suggest common mechanisms of action in their biological pathways. Furthermore, all genes involved in autoimmunity may have promoters with such common characteristics. In contrast, promoters of genes associated with T2D seem to be similar in variability to those found in our genome-wide analysis of promoters. This suggests that the mechanisms involved in T2D are more numerous and less specific.

**Conclusion:** In the future of great interest will be if the same patterns found in T1D will be identified also in other autoimmune diseases. Also, a separate study of obesity associated vs. not associated T2D could further clarify the relationship between these two metabolic disorders.



Clinical Trial Registration Number: CNCS-UEFISCDI, project number PN-II-ID-PCE-2011-3-0429

## 281

### Genetic and expressional evidence for involvement of post-translational modifications as potential triggers of immune intolerance in human islets

A.J. Overgaard<sup>1</sup>, F. Piva<sup>2</sup>, C.A. Brorsson<sup>1</sup>, C.H. Bang-Berthelsen<sup>1</sup>, J. Nerup<sup>1</sup>, F. Pociot<sup>1</sup>, J. Størling<sup>1</sup>

<sup>1</sup>Glostrup Research Institute, Glostrup University Hospital, Denmark,

<sup>2</sup>Polytechnic University of Marche, Ancona, Italy.

**Background and aims:** The loss of immune tolerance to the insulin-producing beta-cells leading to type 1 diabetes (T1D) is unclear. Although several auto-antigens are known, none of these except for insulin are beta-cell-specific and do therefore not explain the specific T-cell-mediated destruction of the beta-cells. We hypothesize that the inflammatory environment in the islets in the initiation phase of T1D pathogenesis induces post translational modifications (PTMs) of beta-cell proteins leading to creation of new antigenic epitopes that are non-immune-tolerant and thus stimulate T-lymphocyte reactivity. We further suggest that part of the genetic susceptibility to T1D may be explained by variation in genes that encode enzymes involved in PTM processes. The aim of this study was to identify genes encoding PTM enzymes in islets that may play a role for induction of beta-cell immune intolerance in T1D.

**Materials and methods:** A comprehensive bioinformatics survey was used to identify all potential proteins involved in selected PTM categories (acetylation, amidation, citrullination, glycosylation, nitrosylation and sumoylation). Genome-wide association scan (GWAS) data was used to map T1D-associated single nucleotide polymorphisms (SNPs) to the genes encoding the identified PTM enzymes. Gene expression of candidates was examined by micro array analysis of human islets exposed for 48 hours to pro-inflammatory cytokines (IL-1+IFN+TNF).

**Results:** We identified a total of 292 genes that encode proteins involved in the six PTM categories. Impressively, 120 of these genes were found to contain T1D-associated SNPs ( $p < 0.05$ ). A Q-Q plot of the results demonstrated a strong enrichment of T1D-associated SNPs in these genes as compared to expected by random chance. Human islet gene expression analysis revealed that 19 of the genes that harboured significant SNPs also were significantly

regulated by cytokines ( $n=4$ ,  $p < 0.05$ ) highlighting that these genes may be of particular interest in the context of regulating PTMs in the islets and beta-cells during inflammatory stress.

**Conclusion:** These observations strongly support a genetic component in T1D risk related to control of PTMs and suggest that the roles of these specific enzymes in islets and pancreatic beta-cells should be further investigated.

*Supported by: EFSD/JDRF/NovoNordisk grant, NNF, The Danish Research Council, The Sehested-Hansen Foundation*

## 282

### Reduced gene expression of killer cell lectin-like receptor subfamily C, member 3, an activating receptor of natural killer cells, in patients with fulminant type 1 diabetes

S. Nakata<sup>1</sup>, A. Imagawa<sup>1</sup>, Y. Miyata<sup>1</sup>, A. Yoshikawa<sup>1</sup>, K. Okita<sup>1</sup>, T. Funahashi<sup>1,2</sup>, S. Nakamura<sup>3</sup>, K. Matsubara<sup>3</sup>, H. Iwahashi<sup>1</sup>, I. Shimomura<sup>1</sup>

<sup>1</sup>Department of Metabolic Medicine, <sup>2</sup>Department of Metabolism and

Atherosclerosis, Graduate School of Medicine, Osaka University, Suita,

<sup>3</sup>DNA Chip Research Inc., Yokohama, Japan.

**Background and aims:** Fulminant type 1 diabetes is an independent subtype within type 1 diabetes that is characterized by the remarkably rapid destruction of pancreatic beta cells. This subtype accounts for approximately 20% of acute-onset type 1 diabetes in Japan and has been reported worldwide, particularly in East Asia. The accumulating lines of evidence suggest that viral infection would contribute to beta cell death, however, the detailed pathophysiology has not been understood. In this study, we tried to clarify the mechanisms involved in the development of fulminant type 1 diabetes by a comprehensive approach using gene expression microarray analysis in peripheral blood cells.

**Materials and methods:** Thirty-two patients with type 1 diabetes (16 fulminant type 1 and 16 classical type 1A) and 9 non-diabetic control subjects were enrolled in this study. All patients had recovered from ketosis at disease onset and had been receiving intensive insulin injection therapy, which means that all patients and controls were free from of metabolic derangements at the time of blood sampling. We performed gene expression microarray (Agilent Whole Human Genome Oligo Microarray 44k (Design ID: 014850), Agilent Technologies, USA) analysis using peripheral blood cells in a small group of subjects, then performed a volcano plot analysis to determine the genes that were differentially expressed between fulminant type 1 diabetes and type 1A diabetes or control. We performed real-time RT-PCR using peripheral blood cells and natural killer (NK)-enriched cells isolated from peripheral blood mononuclear cells (PBMCs). We also determined the proportion of NK cells in PBMCs using flow cytometry.

**Results:** Gene expression microarray analysis revealed that killer cell lectin-like receptor subfamily C, member 3 (KLRC3), which is known as a NK cell activating receptor, reduced expression in fulminant type 1 diabetes compared with both control and type 1A diabetes. Next, we confirmed that the expression of KLRC3 was significantly lower in fulminant type 1 diabetes compared with the controls using peripheral blood cells (i.e., the relative gene expression was  $0.55 \pm 0.26$  and  $1.00 \pm 0.78$  for 16 patients with fulminant type 1 diabetes and 9 controls, respectively;  $p = 0.0412$ ) and NK-enriched cells isolated from PBMCs (i.e., the relative gene expression as  $0.38 \pm 0.30$  and  $1.00 \pm 0.78$  for 15 patients with fulminant type 1 diabetes and 9 controls, respectively;  $p = 0.0108$ ) by real-time RT-PCR. Further study revealed that the proportion of NK cells in PBMCs was significantly lower in fulminant type 1 diabetes than in controls using flow cytometry. The proportion of NK cells in PBMCs correlates with the duration of the onset of fever to hyperglycemic symptoms in fulminant type 1 diabetes.

**Conclusion:** Our study suggests that impairment of NK cell activity is caused by reduced NK activating receptor gene expression and reduced proportion of NK cells in fulminant type 1 diabetes. Impairment of NK cell activity would be associated with the insufficient viral inactivation leading to rapid destruction of pancreatic beta cells in fulminant type 1 diabetes.

*Supported by: KAKENHI (JSPS, MHLW)*

## 283

**Transcriptome analysis of the murine type 1 diabetes locus Idd6.3**U.C. Rogner<sup>1</sup>, B. Lebaillly<sup>1</sup>, C. He<sup>1,2</sup>, P. Avner<sup>1</sup>;<sup>1</sup>URA2578 GMM, Institut Pasteur, <sup>2</sup>Inserm, Paris, France.

**Background and aims:** Our study aims at the characterization of type 1 diabetes loci (Idd) on mouse chromosome 6 in the nonobese diabetic mouse. Idd6 is a locus that controls diabetes development via the activity of CD4 T cells. C3H alleles at Idd6 are protective in diabetes transfer assays. This phenotype has been recently localised to the 700 kb interval of Idd6.3, containing about 10 genes. These include the candidate Aryl hydrocarbon receptor nuclear translocator-like 2 (Arntl2), a homologue of the Arylhydrocarbon receptor. Our current experiments were designed to identify putative downstream targets of Idd6.3 and Arntl2.

**Material and methods:** CD4 T cells were isolated by MACS from three spleens of NOD.C3H congenic females 6.VIIIa (NOD alleles at Idd6.3) and 6.VIIIc (C3H alleles at Idd6.3), pooled and activated for 24 h in the presence of anti-CD3, anti-CD28, and IL2. Three independent biological samples of RNA were then used for microarray analysis (Affymetrix Exon 1.0 ST). Genes with expression differences ( $p < 0.05$  in BH-FDR test) were considered for further analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7.

**Results:** The comparison of the transcriptome of CD4 T cells between two the two Idd6.3 strains showed differences in expression of 99 genes. 69 genes were downregulated in the strain 6.VIIIc compared to 6.VIIIa, whilst 30 genes were upregulated in 6.VIIIc versus 6.VIIIa. Clustering analysis using DAVID at medium stringency revealed 12 functional clusters. These included, in order, ribosomal proteins, cytokines, positive regulation of the immune system, immune defense response, cell migration, hemopoiesis, protein transport, cell fraction, membrane proteins, apoptosis, ion binding, and DNA binding. Interestingly, several immune relevant genes in the interferon pathway appear to be deregulated (see table).

**Conclusion:** These results suggest an implication of the 700 kb Idd6.3 region in the regulation of the expression of several cytokines and antigens. Their deregulation may be associated with decreased diabetogenic activity of the 6.VIIIc derived splenocytes. The data of the transcriptome experiments will also be combined with the NOD and C3H sequence analysis in the Idd6.3 region.

**Immune relevant genes influenced by Idd6.3**

gene	fold change (6.VIIIc/a)	function
IFNg	-1,48	interferon-gamma, immunoregulatory cytokine
IL24	-1,49	interleukin 24, controls cell survival and proliferation
CCL22	-1,41	C-C motif chemokine 22
CD24a	-1,42	CD24 antigen, signal transducer
CD55	-1,56	decay-accelerating factor (DAF), regulates the complement system
LCP2	-1,72	lymphocyte cytosolic protein 2, T cell development and activation
IFITM3	-1,49	interferon-induced transmembrane protein 3, immune defense
CCL19	+1,37	C-C motif chemokine 19, role in lymphocyte recirculation
IFNz	+1,61	interferon zeta/limitin, immunomodulatory cytokine

Supported by: EFSD/JDRF/Novo Nordisk grant, ANR, ARD, CORDDIM

## PS 003 Genetics of type 2 diabetes

## 284

**Parent-of-origin effect of GRB10 variant on insulin and glucose levels during OGTT in the pathogenesis of type 2 diabetes**W. Poon<sup>1</sup>, P. Almgren<sup>1</sup>, A. Stančáková<sup>2</sup>, Y.-C. Cheng<sup>3</sup>, K.D. Silver<sup>3</sup>, A.R. Shuldiner<sup>3</sup>, M. Laakso<sup>2</sup>, L. Groop<sup>1</sup>, V. Lyssenko<sup>1</sup>, for the MAGIC investigators;<sup>1</sup>Department of Diabetes & Endocrinology, Institution for Clinical Sciences in Malmö, Lund University, Malmö, Sweden, <sup>2</sup>Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland, <sup>3</sup>Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, USA.

**Background and aims:** Growth factor receptor-bound protein 10 (GRB10) is a negative regulator of insulin receptor signaling and has previously been implicated in the type 2 diabetes (T2D) pathogenesis. Recently, in the GWAS meta-analysis for insulin secretion traits (MAGIC), we identified a *GRB10* SNP associating with insulin secretion during OGTT. *GRB10* is an imprinted gene and has been shown to have tissue- and isoform-specific expression. Imprinted genes often show different parental transmissions. The aim of our study was to evaluate the parent-of-origin effect (POE) of the *GRB10* variant on insulin and glucose levels during OGTT.

**Materials and methods:** The *GRB10* variant was genotyped in a total of about 2300 trios (child/mother/father) from the Swedish-Finnish Trios, Amish Family Diabetes and EUGENE2 studies. Offspring were grouped according to parental allele transmission (e.g. ApGm = A paternal / G maternal). POE on insulin traits and glucose levels was studied using linear regression analysis.

**Results:** In the combined analyses, the maternally transmitted A-allele was associated with ~15% lower early insulin response to glucose at 30 min (CIR,  $p = 0.015$ ), ~15% lower CIR adjusted for insulin sensitivity index ( $p = 0.013$ ), ~13% lower insulin at 30 min during OGTT ( $p = 0.012$ ), ~14% lower insulin at 30 min adjusted for BMI ( $p = 0.006$ ), ~11% lower area under the insulin curve during OGTT ( $p = 0.007$ ) and ~12% lower total insulin response to glucose (AUCIns/AUCGluc,  $p = 0.002$ ). In the Swedish-Finnish Trios, the maternal A-allele was associated with ~19% lower fasting plasma glucose during OGTT ( $p = 0.001$ ). Preliminary data in the Swedish-Finnish ( $-0.193 \pm 0.059$ ,  $p = 0.001$ ) and EUGENE Trios ( $-0.019 \pm 0.014$ ,  $p = 0.17$ ) also suggest lower fasting plasma glucose level in the offspring with maternal A-allele compared to maternal G-allele.

**Conclusion:** These results demonstrate that the expected effect of reduced insulin secretion on increase in glucose levels was not seen suggesting that the effect of the risk A-allele of the *GRB10* variant could also include other regulators of glucose like glucagon.

Supported by: EFSD/GSK grant, Swedish Research Council, Academy of Finland, NIH research grants

## 285

**Differential effects of type 2 diabetes and fasting glucose/insulin associated loci on insulin secretion stimulated by glucose, GLP-1 or arginine as measured by hyperglycaemic clamps**N. van Leeuwen<sup>1</sup>, G. Nijpels<sup>2</sup>, T.W. van Haeften<sup>3</sup>, P.E. Slagboom<sup>4</sup>, E.J. de Geus<sup>5</sup>, J.M. Dekker<sup>2</sup>, D.I. Boomsma<sup>5</sup>, A. Fritsche<sup>6</sup>, E.M. Eekhoff<sup>7</sup>, L.M. 't Hart<sup>1,4</sup>;<sup>1</sup>Molecular Cell Biology, Leiden University Medical Center, Netherlands,<sup>2</sup>The EMGO institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands, <sup>3</sup>Department of Internal Medicine, Utrecht University Medical Center, Netherlands, <sup>4</sup>Section Molecular Epidemiology, Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Netherlands, <sup>5</sup>Biological Psychology, VU University, Amsterdam, Netherlands, <sup>6</sup>Department of Internal Medicine, Eberhard-Karls University of Tübingen, Germany, <sup>7</sup>Diabetes Center, Department of Internal Medicine, VU University Medical Center, Amsterdam, Netherlands.

**Background and aims:** Currently approximately 65 loci associate with type 2 diabetes, fasting glucose and/or fasting insulin in GWAS and several of these have been associated with beta-cell function and/or insulin resistance, calculated as HOMA-B and HOMA-IR from OGTT. We assessed whether these loci are associated with glucose, glucagon-like peptide 1 (GLP-1) or arginine stimulated insulin secretion during hyperglycaemic clamps, the gold standard for assessment of beta cell function.

**Materials and methods:** Four hundred and fifty volunteers (260 NGT; 190 IGT) underwent a 2-h hyperglycaemic clamp at 10 mmol/l glucose. First- and second-phase glucose-stimulated insulin secretion (GSIS), insulin sensitivity index and disposition index were calculated. A subset had an extended clamp with additional GLP-1 and arginine stimulation ( $n=228$ ). Genetic variation in the 65 loci was assessed using the Cardio-Metabochip. Data from linear regression (GEE) represent results for the best associated SNP in the 50 loci that are well represented on the Cardio-Metabochip. Associations between genetic variation and glucose, GLP1 or arginine stimulated insulin secretion were regarded significant if at least one beta cell measure reached the study wide significance threshold of  $p < 2 \times 10^{-4}$  and for the other beta cell measures at least  $p < 2 \times 10^{-3}$ .

**Results:** Nine T2D and/or fasting glucose/insulin loci were significantly associated with beta cell function. Gene variants in *GIPR*, *CDKAL1*, *ADAMTS9*, *SLC2A2* and *PROX1* showed the largest association with the disposition index and a nominal ( $p < 2 \times 10^{-3}$ ) association with first phase GSIS but not with 2nd phase GSIS, GLP-1 or arginine stimulated insulin secretion. *JAZF1* was associated with both 1st and 2nd phase GSIS but not with the other stimuli. Interestingly, the effect of *FADS1* was only present after arginine stimulation while *DGKB* affected both arginine and GLP-1 stimulated insulin secretion but none was associated with glucose stimulated insulin secretion. Finally *HLA-DQB1* affected the insulin sensitivity index but not beta cell function. Furthermore, another 20 loci showed nominal significant associations with insulin response to one or more of the secretagogues.

**Conclusion:** T2D and fasting glucose/insulin loci are in this study associated with different and specific aspects of beta-cell function measured by hyperglycaemic clamp and three different stimuli. These findings provide further insight into the mechanisms involved in proper functionality of the pancreatic beta-cells and the development of T2D.

Supported by: Dutch Diabetes Research Foundation grant 2006.00.060

## 286

### New insights on the involvement of the intronic SNP rs7903146 on alternative splicing of TCF7L2

M. Pradas-Juni<sup>1,2</sup>, N. Nicod<sup>1,3</sup>, E. Fernández-Rebollo<sup>1,2</sup>, J. Valcárcel<sup>4</sup>, R. Gomis<sup>1,2</sup>;

<sup>1</sup>Diabetes and Obesity Laboratory, IDIBAPS, Barcelona, <sup>2</sup>CIBERDEM, Barcelona, <sup>3</sup>Instituto IMDEA, Madrid, <sup>4</sup>Centre de Regulació Genòmica, Barcelona, Spain.

**Background and aims:** The etiology of type 2 diabetes mellitus (T2D) is complex and involves an interplay between environmental risk factors and susceptible genetic background. To date, several GWAS have strongly correlated the single nucleotide polymorphism, SNP rs7903146 (reference/alternative: C/T) in intron 3 of the transcription factor-7-like 2 (TCF7L2) gene, with an increased risk of T2D. TCF7L2 is a Wnt signaling transcription factor encoded by 17 identified exons, with a complex splicing pattern, giving rise to several isoforms expressed differentially in several tissues including pancreas. One of these isoforms has been associated to a protective effect on  $\beta$ -cell function and survival whereas some others to  $\beta$ -cell apoptosis. Our hypothesis is that T2D individuals carrying the T/T risk genotype express different spliced TCF7L2 transcripts. The main goal was to characterize TCF7L2 spliced variants in immortalized B-lymphocytes, more precisely to study the expression of the different spliced transcripts, to determine the promoter methylation, the possibility of an extra exon in intron 3 and the protein levels.

**Materials and methods:** We used immortalized B-lymphocytes from 8 subjects, 4 controls and 4 diabetic, each group with 2 individuals homozygous for each genotype. To characterize and measure the different transcripts expression, we designed a set of primers covering the spliced transcripts. To determine the promoter methylation we performed a COBRA assay. To analyze the possibility of an extra exon within intron 3, we interrogated the surrounding genomic area of the SNP using two different databases: the Server Developer by Gil Ast (<http://ast.bioinfo.tau.ac.il>) and a database by Hagen Tilgner (CRG); and then we analyzed the predicted acceptor and donor splice sites using NetGene2 software. Finally we studied the total protein levels by immunoblotting.

**Results:** The expression of total mRNA shows differences between T/T controls and diabetic ( $1.57 \pm 0.06$  vs  $0.73 \pm 0.17$ , respectively). Otherwise, our results did not show differences on exons 3-4-5 splicing. We found a decreased expression, in T/T diabetic patients, of the transcripts including the extra nucleotides in exon 7 ( $0.66 \pm 0.13$ , ctrl:  $0.93 \pm 0.39$ ) and exon 9 ( $0.51 \pm 0.05$ , ctrl:  $0.78 \pm 0.07$ ). Finally, we did not find evidence for the expression of the spliced transcript on exons 12-13-17. Because of that, we checked whether a change

in promoter methylation could be related to the expression results, but none were detected. We found that T/T genotype contains motifs that have been associated as splicing repressors, correlating with a skipped exon. On the other hand, the C/C genotype has different motifs identified as splicing enhancers. Therefore, to rule out these predicted exons, we performed qRT-PCR experiments, but we did not find any of the predicted extra exons. Finally, we found lower TCF7L2 total protein levels in individuals carrying the T/T genotype (T/T:  $0.34 \pm 0.08$ ; C/C:  $1.00 \pm 0.16$ ), supporting our hypothesis that this SNP may generate different splicing isoforms and it could induce changes in protein folding and/or function.

**Conclusion:** Our results show that in T2D patients, there is no preference in the splicing pattern of exon 3-4-5, and the inclusion of extra nucleotides at exons 7 and 9 in T/T diabetic patients is correlated with a reduction in total protein levels.

Supported by: 2009SGR-1426

## 287

### A novel cis-regulatory SNP in the GCK locus

M. Claussnitzer<sup>1,2</sup>, M. Fugmann<sup>1</sup>, H. Hauner<sup>1,2</sup>, H. Laumen<sup>1,2</sup>;

<sup>1</sup>Else Kröner-Fresenius-Centre for Nutritional Medicine, Technical University Munich, Freising, <sup>2</sup>Clinical Cooperation Group Nutrigenomics and type 2 diabetes, IEG, Helmholtz Zentrum München, Neuherberg, Germany.

**Background and aims:** The glucokinase (GCK) enzyme is a glucose sensor which is involved in maintaining blood glucose homeostasis. Common variants in the GCK locus have been associated with elevated fasting plasma glucose. The corresponding linkage disequilibrium (LD) block encloses several non-coding SNPs. The aim of this work was to identify a cis-regulatory SNP which might explain the increased risk of elevated fasting plasma glucose at transcriptional level.

**Materials and methods:** NPs located in high LD were bioinformatically analyzed using Genomatix software. Electrophoretic mobility shift assay (EMSA) and reporter gene experiments in the INS1  $\beta$ -cell and the Huh7 hepatocyte cell line were performed to proof the cis-regulatory potential.

**Results:** All SNPs in strong LD ( $R^2 = 0.7$ ; 500 kb) with the GCK tagSNP rs4607517 were bioinformatically analysed. The SNP rs2908289C/T ( $R^2 = 1.0$ ), which is located 4.5 kb upstream of the  $\beta$ -cell specific GCK promoter, was predicted to be cis-regulatory. A differential allele-specific binding of nuclear proteins was shown by EMSA. We proved the cis-regulatory function by reporter gene assays in the INS1  $\beta$ -cell line. The minor T allele revealed a significantly lower luciferase activity compared to the major C allele. In contrast, no allele-specific difference was observed in the Huh7 hepatocyte cell line, pointing to the cell type-specificity in pancreatic  $\beta$ -cells. Moreover, we demonstrated a glucose-dependent regulation of the luciferase constructs in the INS1  $\beta$ -cell line, supporting the physiological relevance of the regulatory element.

**Conclusion:** We identified a novel cis-regulatory SNP in the GCK LD block. The SNP may regulate expression of the endogenous GCK gene in  $\beta$ -cells, and thus confer a higher risk of increased fasting plasma glucose observed in association studies.

Supported by: Else Kröner-Fresenius-Foundation, HMGU Neuherberg

## 288

### Association between rs2943641 polymorphism near IRS1 locus and incident type 2 diabetes is modified by macronutrient intake and sex

U.C. Ericson<sup>1</sup>, G. Rukh<sup>1</sup>, I. Stojkovic<sup>1</sup>, E. Sonestedt<sup>1</sup>, B. Gullberg<sup>2</sup>, E. Wirfält<sup>2</sup>, M. Orho-Melander<sup>1</sup>;

<sup>1</sup>Diabetes and Cardiovascular disease, Genetic Epidemiology, <sup>2</sup>Nutrition Epidemiology, Department of Clinical Sciences in Malmö, Lund University, Malmö, Sweden.

**Background and aims:** The minor T-allele of rs2943641 near IRS1 has been associated with decreased risk of type-2-diabetes, insulin resistance and adiposity in genome-wide association studies. Dietary intake may influence IRS1 phosphorylation and insulin signaling. Studies have indicated sex-specific associations between IRS1 and adiposity, and between carbohydrate intake and incident type-2-diabetes. We aimed to examine interaction between the IRS1rs2943641 polymorphism and macronutrient intakes on body fat% and incident type-2-diabetes in the Malmö Diet and Cancer cohort, and to investigate if such interaction is sex-specific.



**Materials and methods:** In total 15 227 women and 9 614 men, 45–74 years with dietary data and IRS1 genotypes, and without prevalent diabetes, were included. Dietary data was collected with a modified diet history method. During 12 years follow-up, 1567 incident type-2-diabetes cases were identified.

**Results:** The T-allele associated with lower incidence of type-2-diabetes (P for trend=0.003), and in men, with higher body fat% (P for trend=2×10<sup>-5</sup>). We observed 3-way interaction between sex, IRS1 and carbohydrate intake (P=0.01), as well as between sex, IRS1 and fat intake (P=0.01), on incident type-2-diabetes. Among women the T-allele was only associated with decreased risk in the lower tertiles of carbohydrate intake (P for trend=0.01; P for interaction=0.01). In contrast, among men the T-allele was associated with decreased risk in the lowest tertile of fat intake (P for trend=0.01; P for interaction=0.02).

**Conclusion:** Our results indicate sex-specific interactions between IRS1 and macronutrient intakes on incident type-2-diabetes. The IRS1 T-allele associated with decreased risk of type-2-diabetes in women at low carbohydrate intakes, but in men at low fat intakes.

*Supported by: The Swedish Medical Research Council, the Region Skåne*

## 289

### Dietary fat intake and the L162V polymorphism at the PPARA locus modulate glycaemia and type 2 diabetes risk in the D.E.S.I.R. prospective study

A. Lamri<sup>1,2</sup>, I. Porchay-Baldérelli<sup>1,2</sup>, N. Emery<sup>1,2</sup>, N. Bellili<sup>1,2</sup>, O. Lantieri<sup>3</sup>, B. Balkau<sup>4,5</sup>, M. Marre<sup>1,2</sup>, F. Fumeron<sup>1,2</sup>, D.E.S.I.R. study group; <sup>1</sup>Inserm U695, Paris, <sup>2</sup>Université Paris Diderot, Paris, <sup>3</sup>Institut inter Régional Pour la Santé (IRSA), La Riche, <sup>4</sup>Inserm U1018, Villejuif, <sup>5</sup>Université Paris-Sud 11, Villejuif, France.

**Background and aims:** PPARα is a transcription factor involved in lipid metabolism and energy homeostasis. The rare allele V of the L162V polymorphism has been associated with an increased risk of conversion from impaired glucose tolerance to type 2 diabetes. The V allele has a lower transcriptional activity than the frequent allele L in low ligand concentration, and a higher transcriptional activity in high ligand concentration, suggesting an interaction between the L162V genotype and dietary fat intake. Our aim was to study the effect of this interaction on glycaemia and the risk of incident type 2 diabetes in a 9-year prospective cohort drawn from the French general population, the D.E.S.I.R. (Data from an Epidemiological Study on the Insulin Resistance Syndrome) study.

**Materials and methods:** A food frequency questionnaire was completed by each participant to assess the intake of main nutrients including dietary fat. Statistical analyses included logistic regression and analysis of covariance with adjustment for confounding variables.

**Results:** Among low fat consumers, type 2 diabetes incidence was higher in V carriers than in LL homozygotes (OR [CI95%] = 3.12 [1.64–5.88] P = 0.0005, P interaction = 0.005). A higher fat intake was associated with an increased type 2 diabetes risk in LL homozygotes (OR=1.30 [1.03–1.63] P = 0.02) but not in V carriers. Similar interaction effects were found on glycaemia and HbA1c during the follow up. There was also an interaction between L162V genotype and fibrate treatment (P = 0.05): fibrates significantly reduced glycaemia in V carriers only (P = 0.005). Conversely, V carriers tended to have a lower glycaemia than LL homozygotes among subjects treated with fibrates (P = 0.06).

**Conclusion:** The L162V polymorphism of PPARA modulates the effects of dietary fat or fibrate treatment on glycaemia and type 2 diabetes risk.

## 290

### TCF7L2 rs7903146 interacts with dietary fibre intake modifying LDL- and total cholesterol levels

G. Hindy, U. Ericson, E. Sonestedt, M. Orho-Melander; Department of Clinical Sciences, Lund University, Malmö, Sweden.

**Background and aims:** The type 2 diabetes (T2D) susceptibility variant TCF7L2 rs7903146 has previously been reported to interact with dietary fibre and whole grain on incidence of T2D. TCF7L2 is expressed in high levels in intestinal cells and may be involved in GLP-1 secretion. Recently, short-chain fatty acids (colonic fermentation products of fibre), were shown to trigger secretion of GLP-1 from colonic cultures *in vitro*. As in some studies TCF7L2 variant has been associated with cholesterol levels and as dietary fibre

has been associated with lower cholesterol levels, we hypothesized that fibre intake level could interact with TCF7L2 variant and modify association between fibre intake, TCF7L2 and cholesterol levels.

**Materials and methods:** From the Malmö Diet and Cancer Cardiovascular (MDCCV) cohort we included 5,069 individuals (aged 45–73 years) without T2D and cardiovascular disease at baseline. The diet data was based on a 7-day food diary, a 168-items diet questionnaire and a 1-h diet-history interview. Fasting total cholesterol (TC), high density lipoprotein cholesterol (HDL), and triglycerides (TG) were measured and low density lipoprotein cholesterol (LDL) was calculated by Friedewald's formula. Linear regression was used to analyse association between TCF7L2 genotype and the different components of lipid profile in tertiles of dietary fibre adjusting for age, sex, and BMI. Interaction between TCF7L2 genotypes and dietary fibre intake level on components of the lipid profile was analysed by introducing a multiplicative factor in addition to both of these variables to the equation with further adjustment for season, dietary method, and total energy intake.

**Results:** The TCF7L2 T-allele did not associate with any component of the lipid profile in MDCCV. After stratification by fibre intake tertiles, the TCF7L2 T-allele associated with significantly higher TC and LDL in the highest fibre intake tertile (beta = 0.10 and 0.023 mmol/L, p = 0.026 and 0.017, respectively). High fibre intake associated with lower TG levels (beta = -0.02 mmol/L, p = 0.02), but not with TC, LDL or HDL in MDCCV. Association between high fibre intake and lower TG was driven by a significant association among CC genotype carriers (p = 0.009) but not among T-allele carriers. In addition, high fibre intake associated with higher TC and LDL levels among TT genotype carriers (p = 0.03 and 0.004, respectively) but not among C-allele carriers. A formal test of interaction between TCF7L2 genotype and tertiles of fibre intake on TC and LDL revealed significant interactions (p = 0.022 and 0.021, respectively). No significant interactions were observed on HDL and TG levels.

**Conclusion:** Our results suggest that the association between dietary fibre intake and TC, LDL and TG levels is dependent on the TCF7L2 genotype; high fibre intake associated with lower TG among CC genotype carriers but with higher TC and LDL among TT genotype carriers. Interaction between fibre intake and TCF7L2 was significant on TC and LDL levels. Although the mechanism of such interaction remains unknown, our results provide some further evidence that the mechanism by which TCF7L2 rs7903146 affects TCF7L2 function may be modified by dietary fibre intake.

*Supported by: SMRC, Swedish Heart and Lung Foundation, Pahlsson Foundation*

## 291

### The Krüppel-like factor 6-IVS1-27A (KLF6) polymorphism is associated with more favourable postprandial glucose metabolism in subjects at high risk of type 2 diabetes

G.A. Daniele<sup>1</sup>, H.L. Reeves<sup>2</sup>, L. Agius<sup>3</sup>, M. Gaggini<sup>4</sup>, M. Comassi<sup>1</sup>, R. Miccoli<sup>1</sup>, S. Del Prato<sup>1</sup>, A. Gastaldelli<sup>5</sup>; <sup>1</sup>Department of Endocrinology and Metabolism, Pisa University, Italy, <sup>2</sup>Northern Institute for Cancer Research, Newcastle University, UK, <sup>3</sup>Institute of Cellular Medicine, Newcastle University, UK, <sup>4</sup>Department of Surgery, Pisa University, Italy, <sup>5</sup>Institute of Clinical Physiology, National Research Council, Pisa, Italy.

**Background and aims:** We have previously shown that the KLF6-IVS1-27A polymorphism is associated with better hepatic insulin sensitivity and delayed histological progression of non-alcoholic fatty liver disease (NAFLD). We have proposed that KLF6 acts on glucokinase (GCK) by antagonizing GCKR, its negative regulator in liver. The aim of this study was to evaluate the *in vivo* effects of the KLF6-IVS1-27A polymorphism on postprandial hepatic glucose metabolism.

**Materials and methods:** We studied 35 genotyped subjects (Group 1: 29 wild-type GG and Group 2: 6 carriers of AG allele for the polymorphism, KLF6-IVS1-27A, of KLF6 transcription factor gene) by mixed meal test (MMT) with the use of tracers. All subjects were previously screened for glucose tolerance (OGTT). We evaluated fasting and MMT hepatic glucose production, hepatic insulin resistance index (Hep-IR=EGPxINS), oral glucose absorption, MMT glucose clearance and insulin secretion.

**Results:** All subjects were at high risk for type 2 diabetes (either with family history of type 2 diabetes and/or impaired glucose regulation) but had similar OGTT screening fasting and 2h plasma glucose (5.2±0.6 vs 5.1±0.5 mmol/l and 6.2±2.2 vs 5.6±0.9 mmol/l, mean±SD, GG vs. AG, p=ns). The two groups were similar for age (55±12 vs. 56±14 years, z), BMI (28±4 vs. 26±5, Kg/m<sup>2</sup>), HbA1c (6.0±0.4 vs. 5.9±0.5 %), total cholesterol (10.1±3.4 vs. 10.1±2.1

mmol/l), LDL ( $3.2 \pm 1.3$  vs.  $2.9 \pm 0.7$  mmol/l), HDL cholesterol ( $1.5 \pm 0.3$  vs.  $1.6 \pm 0.5$  mmol/l), triglycerides ( $1.2 \pm 0.9$  vs.  $1.5 \pm 1.3$  mmol/l), creatinine ( $61 \pm 7$  vs.  $61 \pm 15$  micromol/l), (GG vs. AG all  $p = \text{ns}$ ). Fasting Hep\_IR was lower in AG as compared to GG ( $304[171]$  vs  $482[545]$   $\mu\text{mol/min/kg}\cdot\text{pmol/l}$ , median [IQR],  $p < 0.05$ ). During MMT, postprandial glucose profiles were lower in AG carriers than GG group due to better peripheral glucose clearance ( $3.5 \pm 1.7$  vs.  $2.6 \pm 0.8$  ml/kg/min<sup>-1</sup>;  $p < 0.05$ , AG vs GG) since mean postprandial insulin and C-peptide concentrations were similar (mean 0–240 min.:  $291 \pm 193$  vs.  $300 \pm 149$  pmol/l and  $2.7 \pm 1.0$  vs.  $2.5 \pm 0.6$  nmol/l;  $p = \text{ns}$ , AG vs GG) as well as mean EGP ( $2.9 \pm 1.5$  vs.  $3.0 \pm 1.3$   $\mu\text{mol/kg}\cdot\text{min}$ ;  $p = \text{ns}$ , AG vs GG) and post hepatic rate of appearance of ingested glucose ( $15.4 \pm 5.3$  vs.  $13.1 \pm 3.5$   $\mu\text{mol/kg}\cdot\text{min}$   $p = \text{ns}$ ; AG vs GG).

**Conclusion:** AG carriers of KLF6-IVS1-27A polymorphism are characterized by lower postprandial glucose levels, better glucose clearance and hepatic insulin sensitivity.

## 292

### Description of haemoglobin variants among a diabetic population in Portugal

M.G. Barata, Z. Peerally, M.A. Silva, J.F. Raposo;  
Diabetes Portugal, Lisbon, Portugal.

**Background and aims:** Haemoglobin variants are the result of mutations in the globin genes which affect the amino acids of the globin protein. Although hundreds of variants have been identified, only a small number of variants are common and have clinical significance. Haemoglobin variants are inherited in an autosomal recessive manner. Therefore, people who are heterozygous for a given variant are said to have a trait or to be carriers and are usually asymptomatic. Those who are homozygous generally have a disease condition. The accuracy of several glycohaemoglobin measurements methods can be adversely affected by the presence of haemoglobin variant trait. An inaccurate value of HbA<sub>1c</sub> may result either in more aggressive treatment (falsely high) leading to increased episodes of hypoglycaemia, or in a under treatment of diabetes (falsely low). Because of the above, when selecting the assay method, laboratories should take into consideration the characteristics of the population being served. The aim of this study was to identify and evaluate the prevalence of haemoglobin variants in patients from an outpatient diabetes centre in Portugal. And by doing so, confirm the suitability of the equipment present in the laboratory, for the determination of haemoglobin A<sub>1c</sub>.

**Materials and methods:** In 2011, 12213 people with diabetes attended the laboratory at APDP to measure their HbA<sub>1c</sub>. The analyses were performed in a HPLC conventional system, which uses principles of ion-exchange high-performance liquid chromatography (HPLC). In partnership with “Centro Hospitalar e Universitário de Coimbra” in Portugal, the samples with haemoglobin variants were analysed in the conventional system  $\beta$ -thalassaemia short program for identification and then confirmed by isoelectric focusing (IEF) or/and solubility test. If necessary for the determination of uncommon variants, were performed sequencing of  $\alpha$  and  $\beta$  globin chains.

**Results:** This study detected 141 patients with haemoglobin variants. Among this variants, Hb S was the more frequent (76%), followed by Hb D (8%), Hb C (5%) and Hb E (1%). Only 2 samples out of the 12213 had an haemoglobin variant for which the equipment was unable to determine the accurate value of HbA<sub>1c</sub>.

**Conclusion:** In 2011 the prevalence of haemoglobin variants in APDP's laboratory was 1,15%. Moreover the equipment proved to be adequate to the population it serves since for only 0,02% of this population it was unable to measure an accurate value of HbA<sub>1c</sub>. This study also allowed to determine the prevalence of haemoglobin variants by regions (in Portugal) and by country birthplace (in immigrants) of the people who attended APDP in 2011. As expected, there is a marked difference between North and South of Portugal. The immigrants with Africa as birthplace, gave a major contribution to the rise of the prevalence, being these variants essentially of the Hb S type.

## 293

### Detection of haemoglobin variants through the analysis of HbA<sub>1c</sub> by HPLC

J.A. Lopez Medina<sup>1</sup>, M. Cortes Rodriguez<sup>2</sup>, B. Perez Nevot<sup>2</sup>, M. Mayor Reyes<sup>2</sup>, A. Peña Agüera<sup>2</sup>, J.M. Garcia Almeida<sup>1</sup>, F. Tinahones Madueño<sup>1</sup>;  
<sup>1</sup>Endocrinología y Nutrición, <sup>2</sup>Laboratory, Hospital Virgen de la Victoria, Malaga, Spain.

**Introduction:** Measurement of glycated hemoglobin (HbA<sub>1c</sub>) in a single blood sample is considered a reliable measure of long-term metabolic control in diabetic subjects. Determination of glycated hemoglobin is superior to glucose measurements and clinical judgment in evaluating diabetic control. Hemoglobinopathies often interfere with HbA<sub>1c</sub> results. Among the different assays, automated liquid-chromatographic (HPLC) techniques have been widely applied. Hb F and others variants (Hb S, HbC, HbD, HbE, HbJ,...) may interfere with the analysis. Nevertheless, improved HPLC techniques have potential advantages over other methods for determining HbA<sub>1c</sub>.

**Objective:** The objective of our study is to detect haemoglobin variants interfering with haemoglobin A<sub>1c</sub> measurements.

**Materials and Methods:** HbA<sub>1c</sub> measurements were performed on ethylenediamine tetra-acetic acid (EDTA) blood samples using cation-exchange HPLC. Chromatograms of samples run in the months of September to December 2011, were checked by a computer program and visually inspected for abnormal patterns suggesting the presence of hemoglobin variants (additional peaks besides Hemoglobin (Hb) A peak or elevated Hb F peak > 10%). The presence of abnormal patterns on the chromatograms were cross-checked using another cation-exchange HPLC.

**Results:** From 5149 diabetic patients, a total of 18 (0,35%) were identified to have hemoglobin variants: 9 (50%) patients were classified as “unknown variants”; 5 patients elevated Hb F (28%), and 4 (22%) patients heterozygous Hb C.

**Conclusion:** Various factors may affect HbA<sub>1c</sub> measurements according to the assay method used, of which hemoglobin variants are one of them. More than 1000 hemoglobin variants have been identified, with many of them being clinically silent. Therefore, a falsely high or low HbA<sub>1c</sub> value caused by the presence of a clinically silent hemoglobin variant may lead to over- or under-treatment of diabetic patients. Cation-exchange high performance liquid chromatography (HPLC) is one of the methods that is vulnerable to the effect of hemoglobin variants on HbA<sub>1c</sub> measurements. A new software used in our hospital allows the detection of a high number of hemoglobine variants and gives notice to doctors automatically in case this happens. Therefore, the laboratory has considered including the the following statement in the results report as a strategy to adopt: “Possible appearance HbA<sub>1c</sub> variation that can alter the results of HbA<sub>1c</sub>”, thereby alerting the clinician to monitor the diabetic patient; and sending unclassified variants for identification by sequencing, for the physician and patient knowledge.

## PS 004 “Omics” in type 2 diabetes

294

### Gene expression data from a genome-wide transcriptomics study improve the prediction of type 2 diabetes: KORA S4/F4 cohort

C. Herder<sup>1</sup>, M. Carstensen<sup>1</sup>, S. Landwehr<sup>2</sup>, K. Heim<sup>3</sup>, W. Rathmann<sup>2</sup>, B. Thorand<sup>4</sup>, C. Meisinger<sup>4</sup>, H.-E. Wichmann<sup>5</sup>, S. Martin<sup>6</sup>, H. Finner<sup>2</sup>, K. Strassburger<sup>2</sup>, T. Meitinger<sup>3,7</sup>, M. Roden<sup>1,8</sup>, T. Illig<sup>9,10</sup>, H. Prokisch<sup>3,7</sup>; <sup>1</sup>Institute for Clinical Diabetology, Düsseldorf, <sup>2</sup>Institute for Biometrics and Epidemiology, German Diabetes Center, Düsseldorf, <sup>3</sup>Institute of Human Genetics, Helmholtz Zentrum München, <sup>4</sup>Institute of Epidemiology II, Helmholtz Zentrum München, Neuherberg, <sup>5</sup>Institute of Epidemiology I, Helmholtz Zentrum München, Neuherberg, <sup>6</sup>West-German Centre of Diabetes and Health, Düsseldorf, Germany, <sup>7</sup>Institute of Human Genetics, Technical University Munich, <sup>8</sup>Department of Metabolic Diseases, University Hospital Düsseldorf, <sup>9</sup>Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, Neuherberg, <sup>10</sup>Hannover Unified Biobank, Hannover Medical School, Germany.

**Background and aims:** Risk prediction for type 2 diabetes remains suboptimal although a number of major clinical risk factors are known. Biomarkers from novel ‘omics’ technologies may be useful to improve the prediction of type 2 diabetes over and above established clinical risk factors. Therefore, the aim of this study was to test the hypothesis that mRNA transcript data from peripheral blood can improve the accuracy of current models for the prediction of type 2 diabetes.

**Materials and methods:** Genome-wide gene expression levels in fasting peripheral whole-blood samples were measured using the Illumina HumanHT-12 v3 Expression BeadChip in 513 initially non-diabetic individuals who participated in the population-based KORA Survey S4 (1999–2001) in Augsburg/Germany. Oral glucose tolerance tests (OGTT) were performed in KORA S4 and in the follow-up survey KORA F4 (2006–2008), and 50 individuals with incident type 2 diabetes were identified. Associations between normalised and log2 transformed transcript levels and incident type 2 diabetes were assessed by logistic regression using Storey’s critical values at a nominal false discovery rate of 0.05 for the correction for multiple testing. The area under the receiver-operating characteristic curve (AUC) was used as measure of discrimination in three prediction models.

**Results:** Expression levels of 93 transcripts were associated with the risk for incident type 2 diabetes after adjustment for age, sex and body mass index (BMI). Based on a step-wise selection algorithm, 13 of these transcripts were included in three prediction models. The inclusion of transcripts improved the AUC of a basic model (model 1: age, sex, BMI, former smoking status, current smoking status, hypertension) from 0.784 to 0.951 ( $P=6 \times 10^{-8}$ ), of a clinical model (model 2: model 1 + fasting plasma glucose, HbA1c) from 0.839 to 0.966 ( $P=8 \times 10^{-7}$ ) and of a clinical model + OGTT (model 3: model 2 + 2-hour plasma glucose) from 0.911 to 0.970 ( $P=2 \times 10^{-5}$ ).

**Conclusion:** We found that expression levels of multiple transcripts in peripheral blood are associated with incident type 2 diabetes. The combination of 13 transcripts provided excellent discrimination between incident cases and controls in our cohort. Further studies and external replications are needed to test the robustness of our data.

*Supported by:* DZD, NGFNplus Atherogenomics (01GS0834), Leibniz Association (SAW)

295

### Common genetic variants of surfactant protein-D (SP-D) and its gene expression in adipose are linked to obesity and type 2 diabetes

N. Pueyo<sup>1</sup>, F.J. Ortega<sup>1</sup>, J.M. Mercader<sup>2</sup>, J.M. Moreno-Navarrete<sup>1</sup>, S. Bonas<sup>2</sup>, J.I. Rodríguez-Hermosa<sup>3</sup>, G. Pardo<sup>4</sup>, M. Serrano<sup>4</sup>, P. Botas<sup>4</sup>, E. Delgado<sup>4</sup>, W. Ricart<sup>1</sup>, F.J. Tinahones<sup>5</sup>, D. Torrents<sup>2</sup>, J.M. Fernandez-Real<sup>1</sup>;

<sup>1</sup>Hospital of Girona, <sup>2</sup>Joint IRB-BSC program on Computational Biology, Institutació Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, <sup>3</sup>Department of Surgery, Hospital of Girona, <sup>4</sup>Hospital Central de Asturias, Oviedo, <sup>5</sup>Service of Endocrinology and Nutrition, Hospital Clínico Universitario Virgen de Victoria de Malaga, Spain.

**Background:** Surfactant protein-D (SP-D) is a primordial component of the innate immunity system intrinsically linked to metabolic pathways. The association of single nucleotide polymorphisms (SNPs) affecting SP-D with inflammation has been recently reported, but there is virtually no data exam-

ining the relation with insulin resistance and type 2 diabetes (T2D). We also sought to evaluate the expression of this collectin in human omental (OM) adipose tissue and in association with measures of regional inflammation and insulin resistance.

**Methods:** We evaluated a common genetic variant of *SP-D* (rs721917, Met<sup>31</sup>Thr) in a sample of T2D patients and non-diabetic controls ( $n=2,711$ ). This SNP and others within the *SP-D* genomic region were also analyzed in the European Caucasian population, *in silico*, by the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC). In a subset of subjects, Circulating ( $n=1,065$ ) and *SP-D* gene expression ( $n=109$ ) in human OM adipose tissue and isolated fat cells were also analyzed in association with measures of inflammation, obesity and glucose tolerance.

**Results:** Case-control analysis showed the significant association of the SNP rs721917 for *SP-D* with circulating *SP-D* and the risk of T2D. GG carriers for this SNP showed decreased *SP-D* concentrations, fasting glucose, and glycated hemoglobin, and 33% ( $p=0.009$ ) lower risk of T2D, estimated under a recessive model. The significance of differences in fasting glucose between groups remained significant after controlling for origin, age, gender, and circulating *SP-D* concentrations ( $p=0.03$ ). Accordingly, this SNP and other *SP-D* SNPs were associated with quantitative measures of glucose homeostasis, insulin sensitivity, and T2D according to the MAGIC data. On the other hand, *SP-D* expression in OM adipose tissue was significantly decreased in obese subjects with T2D were compared to non-obese and non-T2D individuals ( $-47\%$ ,  $p<0.0001$ ). Indeed, OM *SP-D* gene expression correlated with BMI ( $r=-0.34$ ,  $p<0.0001$ ), percent fat mass ( $r=-0.27$ ,  $p=0.004$ ), and insulin resistance, and was positively associated with insulin receptor substrate 1 ( $r=0.23$ ,  $p=0.02$ ) expression in OM adipose tissue.

**Conclusion:** Current data suggests that *SP-D* gene variants are associated with T2D. On the other hand, *SP-D* expression in OM adipose is inversely associated with measures of obesity and insulin resistance.

*Supported by:* MEC (SAF2005-02073), Generalitat (2005SGR00947 and 2005SGR00467), ISCIII

296

### Acute effects of medium-chain saturated fat on postprandial lipaemia, incretins, ghrelin and gene expression in healthy first degree relatives of persons with type 2 diabetes

A. Pietraszek<sup>1</sup>, S. Gregersen<sup>1</sup>, B.L. Langdahl<sup>1</sup>, S.B. Pedersen<sup>1</sup>, J.J. Holst<sup>2</sup>, K. Hermansen<sup>1</sup>;

<sup>1</sup>Department of Medicine and Endocrinology, Aarhus University Hospital,

<sup>2</sup>The Novo Nordisk Foundation Center for Basic Metabolic Research, Department of Biomedical Sciences, The Panum Institute, University of Copenhagen, Denmark.

**Background and aims:** Subjects with type 2 diabetes (T2D) and their relatives (REL) have increased risk of cardiovascular disease. Increased postprandial triacylglycerolaemia (PPL), which is influenced by diet, is an independent risk factor for cardiovascular disease. Little is known about the effects of medium-chain saturated fat on PPL and gene expression in REL. The objective of this study is to test the hypothesis that intake of medium-chain saturated fat causes larger PPL response in REL compared to controls (CON) and has a differential impact on circulating incretins and ghrelin, as well as on gene expression in muscle and adipose tissue of REL and CON.

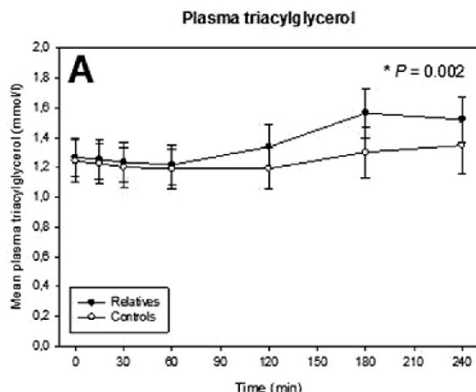
**Materials and methods:** 17 REL and 17 healthy CON with no family history of T2D received a fat-rich meal (79 energy percent from fat) based on medium-chain saturated fat (coconut oil). Plasma concentrations of triacylglycerol, NEFA, insulin, glucose, glucagon-like peptide-1, glucose-dependent insulintropic peptide and ghrelin were measured before the test meal and at regular intervals until 240 min postprandially. Muscle and adipose tissue biopsies were taken at baseline and after the test meal and expression of 31 genes involved in lipid and carbohydrate metabolism was investigated using RT-PCR.

**Results:** Assessed with repeated measurements ANOVA, REL had a higher plasma triacylglycerol response ( $P=0.002$ ) and a tendency towards higher insulin response ( $P=0.100$ ) to the test meal than CON. The responses of plasma glucose, NEFA, glucagon-like peptide-1, glucose-dependent insulintropic peptide and ghrelin did not differ between REL and CON. The genes encoding acyl-CoA long-chain family member 1 (ACSL1), acyl-CoA carboxylase beta (ACACB), hormone-sensitive lipase (LIPE), un-coupling protein 3 (UCP3), solute carrier family 2 (facilitated glucose transporter) member 4 (formerly known as GLUT4), insulin receptor (INSR), phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1), hexokinase 2 (HK2), peroxisome proliferator-activated receptor gamma co-activator 1- alpha (PPARGC1A)



and forkhead box O1 (FOXO1) were up-regulated in response to the meal rich in medium-chain saturated fat in muscle of CON, but not REL.

**Conclusion:** In conclusion, a meal rich in medium-chain saturated fat elicits a larger PPL response, a tendency towards higher insulin response and has a differential impact on gene expression in muscle, but not adipose tissue, of REL compared to CON.



Mean ( $\pm$  standard error of the mean) plasma triacylglycerol in healthy first degree relatives of subjects with type 2 diabetes ( $n=17$ ) and controls ( $n=17$ ) after a meal high in medium-chain saturated fat. Comparison with repeated measurements ANOVA

Clinical Trials Registration Number: <https://register.clinicaltrials.gov> Study ID number: CERN-PPDysMet-AP

Supported by: DanORC and NCoE programme SYSDIET

## 297

### Does DNA methylation of PPARGC1A influence insulin action in first degree relatives of patients with type 2 diabetes?

L. Gillberg<sup>1,2</sup>, S. Jacobsen<sup>1,2</sup>, R. Ribel-Madsen<sup>3</sup>, T.W. Boesgaard<sup>2,4</sup>, O. Pedersen<sup>3,4</sup>, T. Hansen<sup>3,5</sup>, A. Vaag<sup>1,6</sup>,

<sup>1</sup>Department of Endocrinology, Rigshospitalet, Copenhagen, <sup>2</sup>Steno Diabetes Center, Gentofte, <sup>3</sup>The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health Sciences, University of Copenhagen, <sup>4</sup>Hagedorn Research Institute, Gentofte, <sup>5</sup>Faculty of Health Sciences, University of Southern Denmark, Odense, <sup>6</sup>University of Copenhagen, Faculty of Health Sciences, Copenhagen, Denmark.

**Background and aims:** Epigenetics may play a role in the complex pathophysiology of type 2 diabetes (T2D), and increased DNA methylation of the metabolic master regulator peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PPARGC1A*) has been reported in skeletal muscle and pancreatic islets from T2D patients and in muscle from individuals with increased risk of T2D. Our objective was to investigate DNA methylation and gene expression of *PPARGC1A* in skeletal muscle from 124 Danish first degree relatives (FDR) of T2D patients, and to determine the association with insulin action as well as the influence of family relation.

**Materials and methods:** A total of 124 FDR from 46 different families were included. Skeletal muscle biopsies were excised from *vastus lateralis* and insulin action was assessed by modeling of glucose and insulin profiles during oral glucose tolerance tests. DNA promoter methylation and mRNA expression levels were measured using bisulfite sequencing and quantitative real-time PCR, respectively.

**Results:** The average degree of *PPARGC1A* methylation at four different CpG sites was associated with whole body insulin sensitivity in a paradoxical positive manner ( $\beta=0.37$ ,  $P=0.02$ ), and correlated inversely with fasting insulin levels ( $\beta=-0.89$ ,  $P=0.05$ ). DNA promoter methylation was not associated with *PPARGC1A* gene expression. The heritability estimate of *PPARGC1A* gene expression was high ( $h^2=71\pm25\%$  ( $h^2\pm SE$ ),  $P=0.002$ ), suggesting genetic regulation to play a role. No significant effect of familiarity on DNA methylation was found.

**Conclusion:** Increased DNA methylation of the *PPARGC1A* promoter is unlikely to play a major causal role for the development of insulin resistance in FDR of patients with T2D.

Supported by: EUGENE2, FØSU

## 298

### Common variant in SLC47A1 encoding multidrug and toxin extrusion 1 transporter is associated with stronger effect of metformin on glycaemic control in type 2 diabetes

I. Tkac<sup>1</sup>, M. Javorsky<sup>1</sup>, L. Klimcakova<sup>2</sup>, Z. Schroner<sup>1</sup>, M. Fabianova<sup>1</sup>, E. Babjakova<sup>1</sup>, H. Hermanova<sup>2</sup>;

<sup>1</sup>Department Internal Medicine 4, <sup>2</sup>Department of Medical Biology, Safarik University, Kosice, Slovakia.

**Background and aims:** Pharmacogenetic studies revealed several gene variants associated with response to metformin treatment. Variants in genes encoding organic cationic transporters (OCT) OCT1 (encoded by *SLC22A1*), OCT2 (*SLC22A2*) and multidrug and toxin extrusion 1 (*MATE1*) transporter (*SLC47A1*) were shown to be associated with both the pharmacokinetics and glucose-lowering effect of metformin. None of the primary observations has been replicated in patients with type 2 diabetes so far. The aim of the present study was to investigate possible associations of the mentioned gene variants with glycaemic control in patients with type 2 diabetes.

**Material and methods:** 148 drug-naïve patients with type 2 diabetes (72 males/76 females) were included in the present study. Mean age ( $\pm$ SEM) at initiation of metformin treatment was  $57.5\pm0.9$  years, mean baseline  $HbA_{1c}$  was  $7.64\pm0.09\%$ , and mean metformin dose on-treatment was  $1,400\pm40$  mg/day. Genotyping for *SLC22A1* rs612342 A>C, *SLC22A2* rs316019 G>T and *SLC47A1* rs2289669 G>A variants was done using real-time PCR with subsequent melting-curve analysis. The primary outcome of the study was the reduction in  $HbA_{1c}$  ( $\Delta HbA_{1c}$ ) after 6-month metformin treatment.

**Results:** All examined genotypes followed Hardy-Weinberg equilibrium. *SLC47A1* rs2289669 genotype was significantly associated with  $\Delta HbA_{1c}$  [GG ( $n=45$ ):  $0.61\pm0.15\%$ , GA ( $n=74$ ):  $0.52\pm0.11\%$ , AA ( $n=29$ ):  $1.10\pm0.18\%$  ( $p=0.027$ )]. AA homozygotes had twice as high reduction in  $HbA_{1c}$  as the patients carrying G-allele (GG+GA:  $0.55\pm0.09\%$  vs. AA:  $1.10\pm0.18\%$ ,  $p=0.008$ ). The significance of this difference persisted after adjustment for age, gender, BMI, baseline  $HbA_{1c}$ , metformin dose and creatinine in a general linear model ( $p=0.018$ ). Among the covariates, baseline  $HbA_{1c}$  ( $p<0.001$ ) and metformin dose ( $p=0.025$ ) were also significantly associated with  $\Delta HbA_{1c}$ . The entire model explained more than a half of  $\Delta HbA_{1c}$  variance ( $r^2=0.55$ ), and *SLC47A1* genotype explained 4% of the variance. The examined *SLC22A1* and *SLC22A2* variants were not significantly associated with the response to metformin treatment.

**Conclusion:** The present study showed that approximately 20% of diabetic patients who are homozygous for variant A-allele of *SLC47A1* rs2289669 have double reduction of  $HbA_{1c}$  during first six months of treatment with metformin in comparison with G-allele carriers. This study adds further evidence to personalized treatment of patients with type 2 diabetes.

Supported by: VEGA 1/0112/11, VEGA 1/0340/12, Ministry of Education, Slovakia

## 299

### Common genetic variation in the FNDC5 locus, encoding the novel muscle-derived 'browning' factor irisin, determines insulin sensitivity

A.A. Böhm<sup>1,2</sup>, M. Scheeler<sup>3</sup>, L. Berti<sup>3</sup>, J. Machann<sup>2,4</sup>, F. Schick<sup>2,4</sup>, F. Machicao<sup>1,2</sup>, N. Stefan<sup>1,2</sup>, A. Fritsche<sup>1,2</sup>, C. Weigert<sup>1,2</sup>, H. Staiger<sup>1,2</sup>, H.-U. Häring<sup>1,2</sup>, M. Hrabě de Angelis<sup>3</sup>;

<sup>1</sup>Department of Internal Medicine IV, Eberhard Karls University Tübingen, <sup>2</sup>Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Centre Munich at the University of Tübingen, <sup>3</sup>Institute of Experimental Genetics, Helmholtz Centre Munich, German Research Centre for Environmental Health, Munich, <sup>4</sup>Department of Diagnostic and Interventional Radiology, Section of Experimental Radiology, Eberhard Karls University Tübingen, Germany.

**Background and aims:** Recently, the novel myokine irisin was described to drive 'browning' of adipose tissue, to increase energy expenditure, and to improve obesity and insulin resistance in high fat-fed mice. Here, we assessed whether common single nucleotide polymorphisms (SNPs) in the locus of *FNDC5* (fibronectin type III domain-containing protein 5), encoding the irisin precursor, contribute to prediabetic phenotypes (overweight, glucose intolerance, insulin resistance, impaired insulin release) in humans.

**Material and methods:** A population of 1,976 individuals was characterized by oral glucose tolerance tests and genotyped for four tagging SNPs covering all common variants (minor allele frequency  $\geq 0.05$ ) in the *FNDC5* locus. In addition, subgroups of 486, 360, and 305 subjects underwent hyperinsu-

linaemic-euglycaemic clamps, magnetic resonance imaging/spectroscopy, and intravenous glucose tolerance tests, respectively. Glucose, insulin, and C-peptide were measured. From 37 participants, muscle biopsies were obtained for the preparation of human myotube cultures.

**Results:** After appropriate adjustment and Bonferroni correction for the number of tested variants, SNPs rs16835198 and rs726344 were associated with in vivo measures of insulin sensitivity ( $p_{\text{Bonferroni}} < 0.0127$ ). Via interrogation of publically available data from the MAGIC consortium, rs726344's effect on insulin sensitivity (fasting insulin) was replicated ( $p_{\text{MAGIC}} = 0.0167$ ,  $p_{\text{meta-analysis}} = 0.0002$ ). Moreover, human myotube *FND5* expression was negatively associated with appropriately adjusted in vivo measures of insulin sensitivity ( $p \leq 0.0465$ ).

**Conclusion:** This study provides evidence that the *FND5* gene, encoding irisin, influences insulin sensitivity in humans. This data translates recent findings in mice to the human situation and establishes a role of irisin in the pathogenesis of human (pre-)diabetes.

*Supported by: German Federal Ministry of Education and Research (BMBF) to the DZD e.V.*

### 300

#### Association of ACACB rs2268388 G>A polymorphism with metabolic syndrome and type 2 diabetes in a French cohort

F. Fumeron<sup>1,2</sup>, R. Roussel<sup>1,2</sup>, M. Zain<sup>1,2</sup>, A. Lamri<sup>1,2</sup>, N. Bellili<sup>1,2</sup>, N. Emery<sup>1,2</sup>, O. Lantieri<sup>3</sup>, G. Velho<sup>1,2</sup>, B. Balkau<sup>4,5</sup>, M. Marre<sup>1,2</sup>;

<sup>1</sup>UFR de Médecine, INSERM U695, Paris, <sup>2</sup>UFR de Médecine, Univ Paris Diderot, Paris, <sup>3</sup>IRSA, La Riche, <sup>4</sup>INSERM U1018, Villejuif, <sup>5</sup>Univ Paris Sud, Villejuif, France.

**Background and aims:** Fatty acid metabolism is involved in insulin resistance and type 2 diabetes (T2D) pathogenesis. Acetyl-CoA carboxylase  $\beta$ , coded by the ACACB gene, plays a key role in fatty acid synthesis and oxidation pathways. ACACB is expressed at high levels in white adipose tissue. The rs2268388 G>A ACAB polymorphism has been previously associated with obesity and T2D in Spanish women. We investigated the relationship between the rs2268388 G>A ACAB polymorphism and the risk of T2D and/or the metabolic syndrome (MetS) in the DESIR (Data from an Epidemiological Study on the Insulin Resistance Syndrome) cohort, a 9-year prospective study of the French general population.

**Materials and methods:** Over 4000 men and women could be analyzed for the prevalence and the 9 year incidence of T2D and the MetS. The NCEP-ATIII definition of the MetS was used: 1) waist circumference  $> 102/88$  cm for men/women; 2) elevated triglycerides:  $\geq 1.70$  mmol/l; 3) HDL-C:  $\leq 1.03$  mmol/l for men and 1.29 mmol/l for women; 4) blood pressure: systolic blood pressure  $\geq 130$  or diastolic  $\geq 85$  mmHg; 5) fasting glycemia  $\geq 6.1$  mmol/l. The odds ratios to develop T2D and the MetS were assessed separately by logistic regression, adjusting for age, sex, alcohol intake, physical activity, and smoking status, with or without additional adjustment for BMI. The effect of genotype on BMI changes during the follow-up was tested by ANOVA for repeated measures.

**Results:** The A minor allele frequency in the whole DESIR cohort was 0.143. The A allele was associated with a higher T2D incidence (dominant model: OR, 1.51; 95%CI, 1.10–2.09;  $p=0.01$ ). It was also associated with a higher prevalence (OR, 1.29; 95%CI, 1.09–1.53;  $p=0.003$ ) and incidence (OR, 1.48; 95%CI, 1.21–1.82;  $p=0.0002$ ) of MetS. Carriers of the A allele had a significantly higher increase in BMI during the follow-up when compared with GG homozygotes (1.27 vs. 1.10 kg/m<sup>2</sup>,  $p=0.02$ ). After further adjustment on BMI, the genetic association was still significant for MetS (prevalence,  $p=0.002$ ; incidence,  $p=0.0002$ ) but remained only as a trend ( $p=0.07$ ) for T2D.

**Conclusion:** The genetic variation at the ACACB locus has been associated with the susceptibility to type 2 diabetes and metabolic syndrome in a large prospective study. This may be explained only in part by an association with BMI.

### 301

#### Genetic variations in DUSP9 locus and CMIP locus are associated with type 2 diabetes in a Japanese population

M. Imamura<sup>1</sup>, H. Fukuda<sup>2</sup>, M. Iwata<sup>3</sup>, H. Maegawa<sup>4</sup>, H. Watada<sup>5</sup>, H. Hirose<sup>6</sup>, Y. Tanaka<sup>2</sup>, K. Tobe<sup>3</sup>, K. Kaku<sup>7</sup>, A. Kashiwagi<sup>4</sup>, R. Kawamori<sup>5</sup>, S. Maeda<sup>1,5</sup>;

<sup>1</sup>Laboratory for Endocrinology and Metabolism, RIKEN, Center for Genomic Medicine, Yokohama, <sup>2</sup>St. Marianna University School of Medicine, Kawasaki, <sup>3</sup>Toyama University, Toyama, <sup>4</sup>Shiga University of Medical Science, Otsu, <sup>5</sup>Juntendo University, Tokyo, <sup>6</sup>Keio University School of Medicine, Tokyo, <sup>7</sup>Kawasaki Medical School, Kurashiki, Japan.

**Background and aims:** Rs5945326 near DUSP9 on chromosome X was identified as susceptibility locus for type 2 diabetes (T2D) in a meta-analysis of European genome-wide association studies (GWAS). Further, GWAS in South Asian and East Asian populations have identified additional susceptible loci for T2D. To know the roles of these loci in conferring susceptibility to T2D in the Japanese, we examined the association of these variants with T2D in 11,532 Japanese individuals.

**Materials and methods:** We selected 16 single nucleotide polymorphism (SNP) loci, those associations with T2D had not been evaluated in our Japanese population; rs5945326 near DUSP9, 6 SNP loci derived from South Asian GWAS, rs3923113 near GRB14, rs16861329 in ST6GAL1, rs1802295 in VPS26A, rs7178572 in HMG20A, rs2028299 near AP3S2, and rs4812829 in HNF4A, 9 loci from East Asian GWAS including two borderline SNPs, rs7041847 in GLIS3, rs6017317 in FITM2-R3HDM1-HNF4A, rs6467136 in GCC1-PAX4, rs831571 near PSMD6, rs9470794 in ZFAND3, rs3786897 in PEPD, rs1535500 in KCNK16, rs16955379 in CMIP and rs17797882 near WWOX. We genotyped these 16 SNPs for 11,532 Japanese participants (8,449 T2D and 3,083 controls) using multiplex PCR-invader assay. As for rs5945326, females with homozygote and all males were included in the analysis and males were coded as homozygotes. Genotype distribution differences between the case and control groups were analyzed using a logistic regression analysis, and quantitative traits analyses for fasting plasma glucose (FPG), Homeostasis model assessment-Insulin Resistance (HOMA-IR) and HOMA-beta cell function ( $\beta$ ) were performed by multiple linear regression analysis, adjusting age, sex and log-transformed (ln) BMI.

**Results:** Rs5945326-T was significantly associated with T2D and the association reached genome-wide significant level ( $P = 2.21 \times 10^{-8}$ , per homozygote OR = 1.39, 95% CI 1.24 - 1.57). Rs16955379-T was significantly associated with susceptibility to T2D even after Bonferroni's correction ( $P = 2.6 \times 10^{-3}$ , OR = 1.13, 95% CI 1.04 - 1.22). Five SNPs showed nominal association with T2D (rs6017317;  $P = 0.04$ , OR = 1.07, 95% CI 1.003 - 1.15, rs831571;  $P = 7.2 \times 10^{-3}$ , OR = 1.10, 95% CI 1.03-1.18, rs9470794;  $P = 0.029$ , OR = 1.10, 95% CI 1.01-1.20, rs3786897;  $P = 0.011$  OR = 1.09, 95% CI 1.02-1.17, rs178572;  $P = 0.025$  OR = 1.08, 95% CI 1.01-1.16). Remaining 9 SNPs did not show any association with T2D ( $p > 0.05$ ). Rs16955379-T showed modest effects to increase FPG and to reduce HOMA- $\beta$  in control participants (ln FPG;  $P = 0.027$ ,  $\beta = 0.009$ , se = 0.004, ln HOMA- $\beta$ ;  $P = 0.044$ ,  $\beta = -0.055$ , se = 0.027). Other SNPs did not show any association with BMI, FPG, HOMA-IR or HOMA- $\beta$  in controls ( $p > 0.05$ ).

**Conclusion:** We confirmed the association of DUSP9 locus with T2D in the Japanese at genome wide significant level. CMIP locus was also significantly associated with T2D in our Japanese population, although further evaluations for the associations of this SNP as well as remaining 14 loci are required in the Japanese.

*Supported by: JSPS Grants-in-Aid for Scientific Research, Scientific Research (C)23591361*

### 302

#### Mitochondrial DNA depletion and insulin secretion

D.L. Hine<sup>1</sup>, A.E. Brown<sup>1</sup>, L.M. Cree<sup>2</sup>, D. Gunn<sup>3</sup>, L. Brown<sup>3</sup>, P.F. Chinnery<sup>2</sup>, M. Walker<sup>1</sup>;

<sup>1</sup>Diabetes Research Group, Newcastle University, <sup>2</sup>Mitochondrial Research Group, Newcastle University, Newcastle-Upon-Tyne, <sup>3</sup>Discover, Unilever, Bedford, UK.

**Background and aims:** Type 2 diabetes is an age-related condition and is characterised by a progressive decline in insulin secretion. Mitochondria play a key role in energy generation for insulin secretion by way of the electron transport chain. Thirteen polypeptides of the electron transport chain are mitochondrial DNA (mtDNA) encoded. We previously reported a 40–50% age-related decline in mtDNA copy number in human islets isolated from

non-diabetic donors aged from 20 to 80 years. TFAM, mtDNA Transcription Factor A, regulates mtDNA transcription and mtDNA copy number. Using the MIN6 pancreatic beta cell line, we aimed to replicate the percentage decrease in mtDNA copy number that we observed with ageing in human islets, and to explore whether this affected insulin secretion. By targeting TFAM gene expression, we sought to deplete mtDNA copy number in MIN6 cells.

**Materials and methods:** TFAM gene expression was knocked down using siRNA. Seventy two hours post-transfection, cells were stimulated with 3mM or 25mM glucose and subsequent insulin secretion was determined by ELISA. Real-time PCR was used to quantify TFAM knock down, mtDNA copy number, COX1 and Ins1 insulin gene expression. Mitochondrial DNA copy number was measured by comparing the relative expression of mtDNA encoded target gene ND5, with nuclear DNA encoded reference gene GAPDH. TFAM, COX1 and Ins1 insulin gene expression were expressed relative to the reference gene beta-2-microglobulin (B2M). COX1, cytochrome c oxidase subunit 1, is a mtDNA encoded subunit of cytochrome c oxidase (COX, Complex IV) of the electron transport chain. Analyses presented are from 3 separate experiments, each performed in triplicate.

**Results:** Post-transfection, TFAM mRNA levels were depleted by 80% when compared with the scrambled transfection control ( $p < 0.00001$ ). This resulted in a 30–40% reduction in mtDNA levels compared with the control ( $p < 0.01$ ). Following exposure to 25mM glucose, insulin release was decreased in the TFAM knock down cells (mean  $\pm$  SEM:  $4.15 \pm 0.47$  vs  $6.59 \pm 0.57$  ng insulin/ $\mu$ g RNA,  $p < 0.01$ ). The 30–40% decrease in mtDNA observed correlated with a similar 30% decrease in the level of COX1 mRNA compared to the control ( $p = 0.06$ ). We also measured Ins1 insulin gene expression to see whether the decrease in insulin secretion observed was due to a change in Ins1 expression. We found no difference in Ins1 gene expression between TFAM knock down cells and control cells.

**Conclusion:** We found that mtDNA depletion in MIN6 cells to levels seen in human islets with ageing has a direct effect on insulin secretion. This effect on insulin secretion may be due to a defective electron transport chain following a decrease in the mtDNA encoded components. Mitochondrial DNA depletion did not affect insulin gene expression. Strategies to slow islet mtDNA depletion in man could help to preserve insulin secretion and delay the development of Type 2 diabetes.

Supported by: BBSRC & Unilever

### 303

#### Copper regulates beta cells function by modulating the expression of cytochrome-c-oxidase and its copper chaperones

C. Mantzur<sup>1</sup>, G. Aharon-Hananel<sup>1</sup>, L. Romero-Afrima<sup>1</sup>, F. Vernea<sup>1</sup>, T. Aouizerat<sup>2</sup>, A. Saada<sup>2</sup>, I. Raz<sup>1</sup>, S. Weksler-Zangen<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, Diabetes Unit, Hadassah University Hospital, Jerusalem, <sup>2</sup>Department of Human Genetics and Metabolic Diseases, Hadassah University Hospital, Jerusalem, Israel.

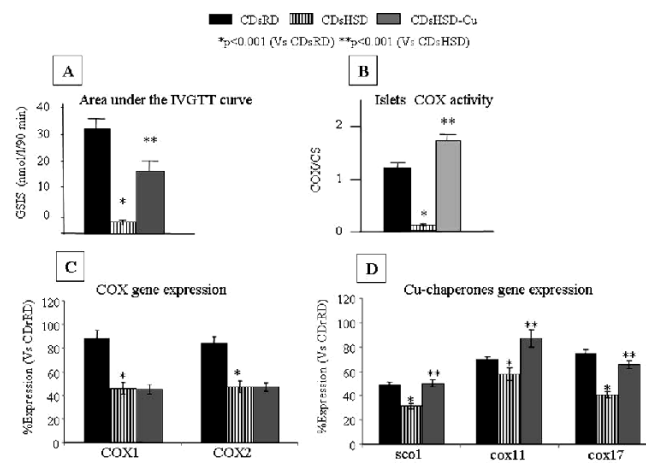
**Background and aims:** Pancreatic beta cells couple glucose oxidative phosphorylation with insulin secretion. Copper (Cu) is a key element in the catalytic activity of the mitochondria-respiratory chain-enzyme cytochrome c oxidase (COX). Cu delivery to COX subunits COX1 and COX2 depends on Cu-chaperones. However, the mechanism underlying the dysfunction of COX in relation to defective glucose stimulated insulin secretion (GSIS) has not been fully established. The Cohen diabetic sensitive (CDs) rat maintains normoglycaemia on regular diet (RD) but exhibits hyperglycemia when fed a high sucrose copper-deficient diet (HSD). Resistant (CDr) rats fed HSD maintained normoglycaemia. Copper supplementation to the HSD (HSD-Cu) restored beta cell function in hyperglycaemic-CDs rats. Therefore, we studied the molecular mechanism underlying the inhibited GSIS induced by copper deficiency in hyperglycaemic-CDs rats.

**Material and Methods:** Hyperglycaemia was induced in CDs rats by a 30 days exposure to HSD (CDsHSD). Hyperglycaemic-CDsHSD rats were fed HSD-Cu (CDsHSD-Cu) for 20 days. Serum insulin levels were measured after overnight fast and during intravenous glucose tolerance test (IVGTT). The expression (qRT-PCR) of COX1, COX2 and the COX Cu-chaperones Sco1, cox17 and cox11 was evaluated in RNA extracted from islets isolated (beta cells) from these rats. Data was normalized according to the expression in CDr rats fed RD (CDrRD) as controls. COX activity measured in islets homogenate was normalised to citrate-synthase (CS), a mitochondrial enzyme that is not part of the respiratory chain and is not copper dependent.

**Results:** The insulin IVGTT area under the insulin curve (GSIS) was significantly reduced in hyperglycaemic-CDsHSD rats compared to normoglycaemic CDrRD rats (Fig 1A). In parallel, islets-COX activity was

markedly reduced in hyperglycaemic-CDsHSD rats (Fig 1B). These correlated with the decrease in expression of COX1, COX2 and the Cu-chaperones in islets isolated from hyperglycaemic-CDsHSD rats (Fig 1C and D). Dietary Cu supplementation restored the GSIS and islet-COX activity of CDsHSD-Cu rats (Figs 1A and B). Cu supplementation induced a corresponding increase in the expression of the Cu-chaperones but failed to restore the expression of COX1 and COX2 (Figs 1C and D).

**Conclusion:** Our study suggests that dietary Cu modulates the gene expression of COX and its Cu-chaperones thereby affecting the capacity of the  $\beta$ -cells to secrete insulin in response to glucose. The differential effect of Cu supplementation on the expression of the COX subunits and Cu-chaperones implies that the CDs-islets may carry an initial inborn deficit in COX expression that cannot be restored by Cu supplementation. COX activity may therefore be rescued by increasing the expression of the Cu-chaperones transporting Cu to COX.



Supported by: Lower Saxony, Hannover, Germany

### 304

#### Decreased activity of cytochrome c oxidase in pancreatic islets and lymphocytes of Cohen diabetes rats: a possible biomarker for prediction of type 2 diabetes

L. Romero-Afrima<sup>1</sup>, A. Saada<sup>2</sup>, C. Matzur<sup>1</sup>, G. Aharon-Hananel<sup>1</sup>, T. Aouizerat<sup>2</sup>, A. Jörn<sup>3</sup>, S. Lenzen<sup>4</sup>, I. Raz<sup>1</sup>, S. Weksler-Zangen<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, Diabetes Unit, Hadassah University Hospital, Jerusalem, <sup>2</sup>Department of Human Genetics and Metabolic Diseases, Hadassah University Hospital, Jerusalem, Israel, <sup>3</sup>Centre of Anatomy and Institute of Clinical Biochemistry, Hannover Medical School, <sup>4</sup>Institute of Clinical Biochemistry, Hannover Medical School, Germany.

**Background and aims:** Pancreatic  $\beta$ -cells couple glucose oxidative phosphorylation with insulin secretion. However, a direct link between dysfunction of cytochrome c oxidase (COX), an enzyme in the mitochondrial respiratory chain and defective glucose stimulated insulin secretion (GSIS) has not been fully established. The Cohen diabetic sensitive (CDs) rat exhibit hyperglycemia when fed a high sucrose copper-deficient diet (HSD) but maintain normoglycemia on regular diet (RD).  $\beta$ -cell dysfunction was restored in CDs rats when HSD was supplemented with copper (Cu), a key element for the catalytic activity of COX. Therefore, we investigated the relation between COX activity and GSIS and compared pancreatic islets to lymphocytes. We hypothesize that COX activity could serve as a biomarker for prediction of type 2 diabetes in humans.

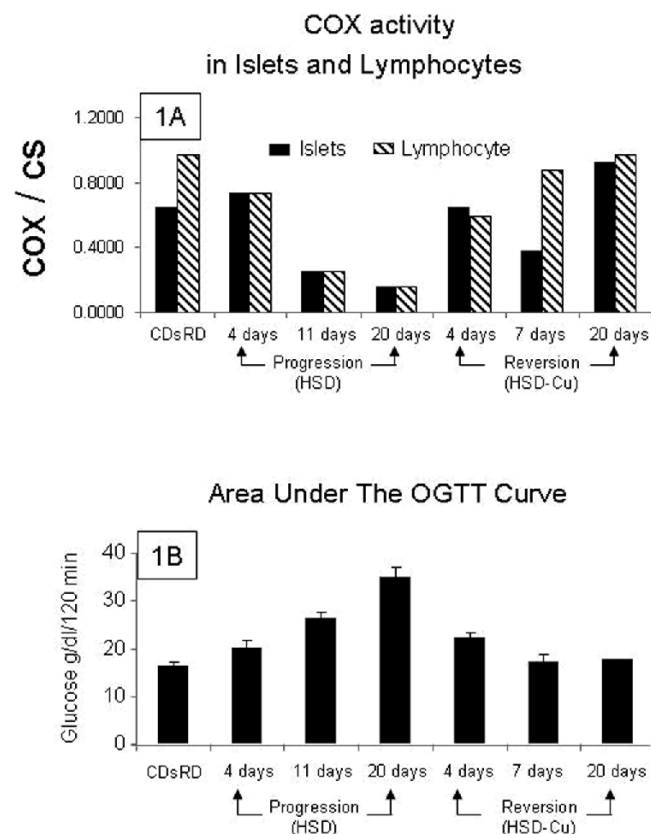
**Material and methods:** CDs rats fed RD (CDsRD) were switched to HSD for 4, 11 and 20 days (Diabetes Progression Study). Hyperglycaemic-CDs rats were fed HSD copper-supplemented (HSD-Cu) for 4, 7, and 20 days (Diabetes Reversion Study). Glucose levels were measured after overnight fast and during oral glucose tolerance test (OGTT). COX activity was measured in homogenates of isolated-islets and lymphocytes. COX activity was normalized to citrate-synthase (CS), a mitochondrial enzyme that is not part of the respiratory chain and is not copper dependent.

**Result:** A parallel change in COX activity was observed in islets and lymphocytes. COX activity decreased in relation to the time on HSD (progression, Fig 1A) in both islets and lymphocytes. Copper supplementation recovered COX activity (reversion, Fig 1A) in relation to the duration on HSD-Cu in



both islets and lymphocytes. The area under the OGTT curve (Fig 1B) increased in relation to the time on HSD (progression) and decreased with time on HSD-Cu (reversion). The increase and decrease in glucose-area under the curve correlated with the reduction and recovery of COX activity in islets and lymphocytes.

**Conclusion:** Our study implies a direct link between low-copper concentrations, reduced COX activity and impaired glucose tolerance in CD rats. The correlation between COX activity in islets and lymphocytes supports COX activity in leukocytes as a biomarker for possible prediction of type 2 diabetes in humans.



Supported by: Lower Saxony, Hannover, Germany

## PS 005 Risk factors for type 2 diabetes

305

### Association of adenovirus 36 infection with obesity and metabolic markers in humans: meta-analysis of observational studies

T. Yamada, K. Hara, T. Kadowaki;

Department of Diabetes and Metabolic Diseases, University of Tokyo, Japan.

**Background and aims:** Several studies have shown that Adenovirus 36 (Ad36) influences the risk of obesity in humans. Clarifying the relationship between Ad36 infection and obesity could lead to more effective approaches for the management of obesity. The objective of this study was to conduct a meta-analysis to confirm the influence of Ad36 infection on obesity and metabolic markers.

**Materials and methods:** We searched MEDLINE and the Cochrane Library for pertinent articles (including their references) published between 1951 and January 28, 2012. Only English language reports of original observational studies were included in this meta-analysis. Data extraction was performed independently by two reviewers. Weighted mean differences (WMDs) and pooled odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated using the random effects model.

**Results:** Of 225 potentially relevant studies, 10 cross-sectional studies (n=2,870) conformed to the selection criteria. Pooled analysis showed that the WMD for BMI of Ad36 infection compared with non-infection was 3.19 (95% CI: 1.44-4.93; P<0.001). Sensitivity analysis restricted to studies of adults yielded a similar result of 3.18 (95% CI: 0.78-5.57; P=0.009). The increased risk of obesity associated with Ad36 infection was also significant (OR: 1.9; 95% CI: 1.01-3.56; P=0.047). No significant differences were found in relation to total cholesterol (P=0.83), triglycerides (P=0.64), HDL (P=0.69), blood glucose (P=0.08), waist circumference (P=0.09), and systolic blood pressure (P=0.25).

**Conclusion:** Ad36 infection was associated with the risk of obesity and weight gain, but was not associated with abnormal metabolic markers including waist circumference. It suggests that Ad36 infection is more associated with accumulation of subcutaneous fat than that of visceral fat. The relationship between Ad36 and obesity should be assessed by further studies, including well-designed prospective studies, to gain a better understanding of whether Ad36 plays a role in the etiology of human obesity.

306

### The SHBG genotype rs1799941 is associated with insulin secretion and carotid atherosclerosis, independently of insulin sensitivity, in a healthy European population

F. Bonnet<sup>1</sup>, M. Laville<sup>2</sup>, B. Balkau<sup>3</sup>, E. Ferrannini<sup>4</sup>, M. Walker<sup>5</sup>, for the RISC investigators;

<sup>1</sup>Dept. of Endocrinology-Diabetology, University hospital, Rennes, France,

<sup>2</sup>INSERM U870-INRA 1235, Centre de Recherche en Nutrition Humaine

Rhône-Alpes, Lyon, France, <sup>3</sup>INSERM CESP U1018, Villejuif, France,

<sup>4</sup>University of Pisa, Department of Internal Medicine, Pisa, Italy, <sup>5</sup>University of Newcastle upon Tyne, Glasgow, UK.

**Background and aims:** Studies applying the Mendelian randomization approach suggest that sex hormone-binding globulin (SHBG) may be involved in the pathogenesis of type 2 diabetes. The SHBG A allele at rs1799941 is associated with an increase in plasma SHBG concentration and a reduced risk of type 2 diabetes. However, the underlying mechanisms remain poorly identified. The aim of our study was to explore whether this SHBG variant is associated with insulin secretion and early atherosclerosis after taking into account the degree of insulin sensitivity, in a cohort with a detailed phenotypic characterisation of glucose metabolism and assessment of carotid intima-media thickness (IMT).

**Materials and methods:** We studied 1097 healthy individuals from the RISC study (Relationship between Insulin Sensitivity and Cardiovascular disease) for whom the SHBG genotype was available. All participants had a euglycemic-hyperinsulinemic clamp and an OGTT with determination of various indices of insulin secretion and an ultra sound recording of the carotid arteries, with a centralised evaluation of IMT.

**Results:** Clamp-assessed insulin sensitivity (M/I value) did not differ according to the SHBG rs1799941 genotype. The insulinogenic index [(Insulin<sub>30</sub>

$\frac{\text{min-Insulin}_{0 \text{ min}}}{(\text{Glucose}_{30 \text{ min}} - \text{Glucose}_{0 \text{ min}})}$ ), which represents early phase insulin secretion, was significantly higher in those who carried the SHBG A allele at rs1799941 [ $111.4 \pm 168.8$  for A/G or A/A ( $n=437$ ) vs  $82.6 \pm 192.5$  pmol/mmol for GG genotype ( $n=594$ ),  $p=0.001$ ]. The disposition index (insulinogenic index  $\times$  M/I value), which estimates early insulin secretion after taking into account for insulin sensitivity, was also significantly increased in the presence of the A allele ( $15.3 \pm 25.2$  for A/G or A/A vs  $10.3 \pm 41.4$  I<sup>2</sup>/mmol<sup>2</sup> for the GG genotype,  $p=0.001$ ). The association between the A allele and increased insulin secretion, as assessed with various indices, persisted after adjustment for age, sex, recruitment centre, waist, physical activity, smoking ( $p=0.008$  for the disposition index). These associations were not modified after adjustment for insulin sensitivity (M/I value). In addition, carotid IMT was lower in those who carried the A allele ( $1.92 \pm 0.3$  for A/G or A/A vs  $1.86 \pm 0.3$  mm for GG genotype,  $p=0.001$ ). The A allele was associated with a reduced risk of having an increased intima media thickness (IMT), highest vs lower three quartiles (OR: 0.70; 95% CI: 0.53–0.93,  $p=0.01$ ), independently of conventional risk factors including LDL-c, glycaemia and blood pressure.

**Conclusion:** In a healthy population, the SHBG variant rs1799941 was associated with enhanced  $\beta$ -cell function and a reduced risk of carotid atherosclerosis, independently of insulin sensitivity. The impact of this SHBG variant on cardiovascular outcomes needs to be further studied in diabetic patients.

Supported by: EC

### 307

#### Is air pollution related to metabolic control in type 2 diabetes?

T. Tamayo<sup>1</sup>, W. Rathmann<sup>1</sup>, U. Krämer<sup>2</sup>, D. Sugiri<sup>2</sup>, M. Grabert<sup>3</sup>, R.W. Holl<sup>3</sup>;

<sup>1</sup>Institute of Biometrics and Epidemiology, German Diabetes Center,

<sup>2</sup>Institut für Umweltmedizinische Forschung (IUF), Düsseldorf, <sup>3</sup>Institute for Epidemiology and Medical Biometry, University Ulm, Germany.

**Background and aims:** Air pollution is a major environmental health problem. Evidence is growing that air pollutants are associated with type 2 diabetes (T2DM) risk. Subclinical inflammation may be a mechanism linking air pollution with T2DM. The aim was to investigate if air pollution contributes to higher HbA1c levels in persons with T2DM in an ecological study.

**Material and methods:** Nationwide regional levels (postal areas) of particulate matter with a diameter of 10 $\mu$ m (PM10) were obtained from background monitoring stations in Germany in 2009 (Umweltbundesamt II 4.2). Mean HbA1c (2009) was calculated in 12,058 newly diagnosed T2DM patients (age:  $64 \pm 14$  yrs; males: 53%) throughout Germany (DPV-database). Mean HbA1c stratified for air pollution quartiles (PM10  $\mu$ g/m<sup>3</sup>) was estimated using linear regression modeling adjusting for age, sex, BMI, diabetes duration, geographic region, in/outpatient treatment, and deprivation (low education, migration).

**Results:** Patients exposed to low PM10 concentrations (Q1) had significantly lower levels of HbA1c than in higher quartiles of exposure (Q2–Q4) even after adjustment of established risk factors (Table). The difference between lowest and top quartile was more pronounced in males. In males, HbA1c levels increased with quartile increment of exposure. Similar results were found after further adjusting for antidiabetic treatment (oral agents, insulin).

**Conclusion:** Because of the possibility of residual confounding (socioeconomic status) and the ecological study design, the evidence for a causal association between air pollution and glycemic control T2DM is suggestive but not sufficient. However, in light of the large worldwide number of people exposed to outdoor air pollution this association deserves further experimental and epidemiological investigations. Table: Mean HbA1c (%) (SD) in type 2 diabetes patients stratified by regional particulate matter (PM10) exposure (quartiles)

Model	Females	Males	Total sample
Q1 <16.4 $\mu$ g/m <sup>3</sup>	6.4 (0.2) <sup>a,b,c</sup>	7.0 (0.3) <sup>a,c</sup>	6.7 (0.2) <sup>a,b,c</sup>
Q2 16.4–<18.05 $\mu$ g/m <sup>3</sup>	6.6 (0.2) <sup>a</sup>	7.2 (0.3) <sup>a,c</sup>	6.8 (0.2) <sup>a</sup>
Q3 18.05–<21.1 $\mu$ g/m <sup>3</sup>	6.6 (0.2) <sup>b</sup>	7.1 (0.3) <sup>f</sup>	6.8 (0.2) <sup>b</sup>
Q4 $\geq 21.1$ $\mu$ g/m <sup>3</sup>	6.6 (0.2) <sup>c</sup>	7.4 (0.3) <sup>c,e,f</sup>	6.9 (0.2) <sup>c</sup>

Abbreviations: Q=Quartile

<sup>a</sup> $p < 0.05$  comparing Q1 and Q2

<sup>b</sup> $p < 0.05$  comparing Q1 and Q3

<sup>c</sup> $p < 0.05$  comparing Q1 and Q4

<sup>d</sup> $p < 0.05$  comparing Q2 and Q3

<sup>e</sup> $p < 0.05$  comparing Q2 and Q4

<sup>f</sup> $p < 0.05$  comparing Q3 and Q4

Supported by: BMBF Competence Network for Diabetes mellitus

### 308

#### Associations between parental longevity and glucose regulation of the offspring: cross-sectional and longitudinal analyses in the KORA S4/F4 Study

B. Kowall<sup>1</sup>, W. Rathmann<sup>1</sup>, A. Peters<sup>2</sup>, B. Thorand<sup>2</sup>, C. Meisinger<sup>2</sup>;

<sup>1</sup>German Diabetes Center, Düsseldorf, <sup>2</sup>Helmholtz Zentrum München, Neuherberg, Germany.

**Background and aims:** Type 2 diabetes is less prevalent in the offspring of centenarians and nonagenarian siblings. We aimed to assess whether this reduction is also found when less extreme criteria of longevity (lifespan of at least 80 years) are applied, and, therefore, assessed associations between parental longevity and the prevalence of prediabetes and diabetes as well as the incidence of diabetes.

**Materials and methods:** Baseline and 7-year follow-up data of 55–74 year old participants of the population-based German KORA S4/F4 cohort study were used for analyses. Subjects whose parents had died due to traumatic causes were excluded. Diabetes was assessed by validated physician diagnoses or by oral glucose tolerance tests. Using logistic regression models, adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated for the associations between parental longevity and glucose regulation at baseline (normal glucose tolerance, prediabetes, diabetes), and incident diabetes, respectively.

**Results:** In age- and sex-adjusted models, diabetes prevalence was lower in subjects with one (OR=0.63, 95%-CI: 0.43–0.93), or two long-lived parents (OR=0.46, 95%-CI: 0.25–0.85). For paternal, but not for maternal longevity, an association with the prevalence of prediabetes was observed (OR=0.72, 95%-CI: 0.52–0.99). Incident diabetes was lower in subjects with two than in subjects without long-lived parents, albeit not significantly (OR=0.76, 95%-CI: 0.33–1.72).

**Conclusion:** Prevalence of type 2 diabetes is strongly reduced in subjects whose parents died at age 80 or later. We found an association between paternal longevity and prevalence of prediabetes but no association between parental longevity and incident diabetes.

Supported by: German Research Foundation project

### 309

#### Socioeconomic inequalities in the prevalence of diabetes in Italy

M. Maggini<sup>1</sup>, V. Minardi<sup>1</sup>, V. Possenti<sup>1</sup>, N. Bertozzi<sup>2</sup>, G. Carrozzi<sup>3</sup>,

L. Sampaolo<sup>3</sup>, L. Bolognesi<sup>3</sup>, PASSI coordinating group;

<sup>1</sup>Epidemiology, National Institute of Health, Roma, <sup>2</sup>Local Health Unit, Italy,

<sup>3</sup>Local Health Unit, Modena, Italy.

**Background and aims:** An inverse relationship between socioeconomic status and prevalence of diabetes, as well as for incidence and mortality, has been shown. Furthermore, persons belonging to disadvantaged social classes are more likely to adopt life styles harmful for health. This work is aimed at determine and quantify inequalities in diabetes mellitus for Italian men and women using data of the Italian Behavioural Risk Factor Surveillance System (PASSI).

**Materials and methods:** The PASSI system is a cross-sectional health survey that was begun in 2007 to provide information on the health status of Italian adult population, through the systematic and continuous collection of data on lifestyles, chronic diseases, socio-demographic variables and adherence to preventive programs. 93% of Italian Local Health Units (LHU), covering about 85% of Italy's 18–69-year-old population, participates in the surveillance. LHU residents (18–69 years) are randomly selected, and trained LHU personnel administer telephone interview using standardized questionnaire. A total of 94,996 persons were interviewed in the period 2007–2009. All statistical analyses were conducted weighting for the whole LHU population. Prevalence Rate Ratios (PRR) were estimated through a multivariate Poisson model. All the analyses were performed separately for men and women.

**Results:** Among the population aged 35–69 years, 6.9% reported to have diabetes (men: 7.7%; women: 6.2%). The prevalence increases with worsening of the socioeconomic conditions: for both men and women the prevalence was higher in people with lower education (men: 16.1%; women: 14.4%), and with economical difficulties (men: 11.9%; women: 11.7%). Diabetes prevalence displays significant geographical variations: 6.5% for men in the North, and 9.3 in the South; 4.6% for women in the North and 8.0% in the South. The multivariate model confirmed a significant association of diabetes with age, gender, education, geographical area and economic difficulties. The PRR for a woman with low education (no education/primary school) vs. high edu-

cation (secondary school/university) was 1.9 (CI 95% 1.6–2.3), and PRR for many difficulties vs. none was 2.1 (CI 95% 1.8–2.6). The PRR for men with low education vs. high education was 1.5 (CI 95% 1.3–1.7), and PRR for many difficulties vs. none was 1.5 (CI 95% 1.2–1.8).

**Conclusion:** A strong association was confirmed between prevalence of diabetes and socio-demographic characteristics, including economic level and geographical area. The highest prevalence was observed in Southern Italy and in disadvantaged social groups. Socioeconomic differences in diabetes are larger among women than in men. The large inequalities found for diabetes prevalence require special attention in equity-oriented research and policies. *Supported by: National Institute for Health, Migration and Poverty (NIHMP)*

### 310

#### Validation of indices predicting hepatic steatosis by <sup>1</sup>H-magnetic resonance spectroscopy and their correlation with glucose tolerance

S. Kahl<sup>1</sup>, R. Livingstone<sup>1</sup>, B. Nowotny<sup>1</sup>, B. Klüppelholz<sup>2</sup>, K. Strassburger<sup>2</sup>, J.-H. Hwang<sup>1</sup>, G. Pacini<sup>3</sup>, A. Gastaldelli<sup>4</sup>, G. Giani<sup>2</sup>, M. Roden<sup>1</sup>; <sup>1</sup>Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf, Germany, <sup>2</sup>Institute for Epidemiology, German Diabetes Center, Düsseldorf, Germany, <sup>3</sup>Metabolic Modeling Unit, National Research Council Institute of Biomedical Engineering, Padova, <sup>4</sup>National Research Council Institute of Clinical Physiology, Pisa, Italy.

**Background and aims:** Hepatic steatosis [fatty liver (FL)] is strongly associated with type 2 diabetes. The Fatty Liver Index (FLI) and the Hepatic Steatosis Index (HSI), algorithms based on laboratory and anthropometric parameters assessed in daily routine, are increasingly used to predict FL in clinical studies. Therefore, solid validation is mandatory. Validation of FL has been performed by ultrasound, which provides qualitative but not quantitative assessment of hepatocellular fat content (HCL). Thus, we tested the accuracy of FLI and HSI by validation of the previously established limits of prediction (values <30 exclude; >60 and >36, respectively, define FL) against exact quantification of HCL by <sup>1</sup>H-magnetic resonance spectroscopy (<sup>1</sup>H-MRS) with HCL concentrations exceeding 5.56% indicating FL. As increased HCL has been shown to associate with impairment of glucose tolerance, we analyzed the relationship of FLI and HSI with glycemia and parameters related to insulin sensitivity (OGIS) and  $\beta$ -cell function (insulinogenic index (IGI)). **Materials and methods:** We studied 98 humans without known diabetes (61f/37m; mean age 58 $\pm$ 12 years; body mass index 26 $\pm$ 4 kg/m<sup>2</sup>) before and during a 75 g oral glucose tolerance test (OGTT) and glucose and insulin were determined in 30min intervals. HCL was measured by <sup>1</sup>H-MRS on a 3T magnet (Philips, Netherlands) using stimulated echo acquisition mode (STEAM) sequence and OGIS (ml/min/m<sup>2</sup>) and IGI (pmol<sub>INS</sub>/mmol<sub>GLUC</sub>) were calculated by validated mathematical model analysis from OGTT glucose and insulin values. Correlations were done using Spearman's rank correlation analysis and OGIS was analyzed using linear regression.

**Results:** Employing MRS, 18 out of 98 subjects had FL. HCL ranged from 0.03% to 39.01% with a median of 2.1% [95% confidence intervals: 0.6; 4.2]. FLI and HSI ranged from 1.6 to 91.4 and 23.9 to 51.5 with a median of 23.1 [9.3; 50.5] and 33.3 [29.7;36.4]. Subjects with FL had markedly lower OGIS (p<0.003) and slightly higher IGI (p<0.02) than subjects without FL. HCL correlated with fasting and 2-h glucose (both r=0.29, p<0.004), OGIS (r=-0.46, p<0.0001) and IGI (r=0.22, p<0.04). Using the proposed cut-off limits, FLI yielded a sensitivity (Se) of 78% and a specificity (Sp) of 83%, HSI a Se of 100% and Sp of 75%. Both indices correlated with HCL (r=0.48 and 0.44, both p<0.0001). Furthermore, FLI and HSI positively relate to fasting and 2-h glucose (r=0.37/0.22, p<0.0002/0.03 and r=0.32/0.22, p<0.001/0.03) and negatively relate to OGIS (r=-0.66/-0.55, both p<0.0001), but only HSI related to IGI (r=0.26, p<0.02). Separate regression analyses showed that after adjustment for age, sex and HCL, both indices were inversely and independently associated with OGIS ( $\beta$ =-1.1, p<0.0001 and  $\beta$ =-4.3, p<0.001).

**Conclusion:** Exact quantification of liver fat content revealed that both indices offer reasonable efficacy to detect steatosis. Independently of HCL, FLI and HSI associate with a measure of insulin resistance. Thus, these indices can serve as valuable predictors of hepatic steatosis and insulin resistance in larger clinical studies.

### 311

#### Can liver enzymes predict progression from prediabetes to type 2 diabetes independent of factors associated with the metabolic syndrome in White Europeans and South Asians?

S.A. Mostafa, K. Khunti, D.H. Morris, D. Webb, B.T. Srinivasan, M.J. Davies; Leicester Diabetes Centre, University of Leicester, UK.

**Background and aims:** Liver enzyme activities have been shown to predict incident Type 2 Diabetes (T2DM) in the general population independent of risk factors in the metabolic syndrome. However their role in predicting progression from prediabetes (PDM) to T2DM has not been investigated. Also it is debated whether ALT or GGT is a better predictor and at which level. Both enzymes independently predict T2DM in their normal physiological range, however for ALT this occurs mainly in Europeans and only in the abnormal range for certain ethnic minority groups. As this has never been investigated in South Asians (SA), we analysed a UK multi-ethnic cohort of White Europeans (WE) and SA with PDM undergoing annual re-screening.

**Materials and methods:** Analysis of 735 participants with PDM (impaired glucose tolerance and/or impaired fasting glycaemia using WHO 1999 criteria) detected during the ADDITION-Leicester screening study, who underwent an annual follow up oral glucose tolerance test between 2005 to 2010. People with baseline ALT or GGT levels 3 times the upper reference limit were excluded. Cox proportion models were used to calculate risk of T2DM in WE (n=538) and SA (n=197) for quartiles of baseline ALT and GGT, adjusting for baseline variables: age, sex, BMI, waist circumference, systolic and diastolic BP, creatinine, deprivation level, smoking status, alcohol intake (units/week), triglycerides and HDL. A further model was tested in a sub-sample of 332 participants from the total cohort including additional adjustments for HOMA-IR, hs-CRP and ethnicity.

**Results:** Over a median of 3 years (range 1-5 years), 48 (8.9%) and 34 (17.3%) WE and SA developed T2DM. Compared to lowest ALT quartile<18IU/L (reference), adjusted hazard ratios (HR) predicting DM for groups 18-23, 24-35 and  $\geq$ 36 were 1.2(0.3-5.3), 2.3(0.6-8.6) and 7.0(2.0-24.7, p<0.005) respectively in WE; trend across groups p<0.001. In SA, compared to <16IU/L (reference), quartiles of 16-20, 21-30 and  $\geq$ 31 produced HR of 0.4(0.1-10.4), 0.1(0.03-0.5) and 0.5(0.1-1.5); trend p<0.05. Within ALT quartiles 1 to 4 in South Asians, the % progression to T2DM was 30.2%, 23.1%, 11.3% and 24.5% respectively (p=0.086). For GGT quartiles, compared to <21IU/L, HR for 21-27IU, 28-40 and  $\geq$ 41 were 3.0 (0.6-14.7), 2.7 (0.5-13.1) and 4.8 (1.0-22.7, p<0.05) in WE, trend p=0.201. In SA, the same analysis was not significant. Further modelling on the sub-sample population revealed the significant trends of predicting progression remained after adjustment for hs-CRP, HOMA-IR and ethnicity for ALT (p<0.05) but not GGT.

**Conclusion:** In WE with PDM, ALT was a stronger predictor of progression to T2DM over 3 years than GGT, independent of factors associated with the metabolic syndrome, insulin resistance, sub-clinical inflammation and alcohol intake. Also the risk of DM was significant in the highest ALT quartile which consisted of levels in the 'high-normal' and abnormal range. However in South Asians, increasing ALT and GGT did not appear to be related to higher progression to T2DM.

### 312

#### Liver fat content is associated with impaired glucose profile in Chinese middle-age and elderly population: Shanghai Changfeng Study

X. Li<sup>1</sup>, M. Xia<sup>1</sup>, H. Ma<sup>1</sup>, H. Lin<sup>1</sup>, W. He<sup>2</sup>, X. Gao<sup>1</sup>;

<sup>1</sup>Fudan University, Department of Endocrinology and Metabolism, <sup>2</sup>Fudan University, Department of Ultrasonography, Shanghai, China.

**Background and aims:** Previous studies have shown that people with NAFLD are more than twice as likely to have T2DM, and even moderate elevation in alanine aminotransferase levels, a poor surrogate of fatty liver, was found to be associated with high-normal glucose levels. Moreover, prospective studies suggest people with NAFLD are more likely to have T2DM at all time points of follow-up. In view of several lines of evidence, it suggests that the deposition of fat in the liver might play a role in the development of T2DM. However, It is unknown to what extent liver fat content (LFC) reflects a defect in impaired glucose profiles. Therefore, in the present study we investigated whether LFC measured by quantitative ultrasonography is associated with glycemic abnormalities in people without prior known T2DM, and further to determined to what extent LFC contribute to the adverse glucose profiles.

**Materials and methods:** We conducted a community-based study among 1608 (596 men and 1012 women) residents without prior known diabetes



mellitus at least 45 years old Changfeng community in Shanghai, China. A standard interview (included life style, diseases history through questionnaires), anthropometrics, laboratory parameters (including serum lipid, fasting blood glucose (FBG), 2h postload plasma glucose (PPG) after oral glucose tolerance test were conducted for each participant). The pictures with legible liver and renal were used to calculate LFC. The independence of the associations of prediabetes and diabetes with LFC was assessed by the multivariate logistic regression analysis. Receiver operating characteristic (ROC) curve analyses were used to determine the appropriate cut-off values for LFC to identify pre-diabetes and diabetes. SPSS 17.0 for Windows was used to perform statistical analyses. All statistical tests were two-tailed, and p-values less than 0.05 were considered as significant.

**Results:** By logistic regression analysis, 1% LFC increment independently predicted prediabetes and diabetes (OR 1.034, 1.019–1.050,  $P < 0.001$ ; 1.025, 1.007–1.044,  $P = 0.007$ , respectively) after adjustment for age, sex, ALT, BMI, WHR, SBP, DBP. Cut-off values were calculated for LFC in association with increases in the prevalence of prediabetes and diabetes using ROC analysis (Fig. 1). The areas under the curve were 0.621, 0.635 ( $P < 0.001$ ). The cut-off values for diagnosis of prediabetes and diabetes were 9.99% and 11.54% for LFC respectively. The sensitivity and specificity of the LFC cut-off value for prediabetes and diabetes was 44.8%, 47.7% and 77.6%, 80.4% respectively.

**Conclusion:** These results suggest that LFC is an independent risk factor for prediabetes and diabetes. The optimal cutoffs of LFC for pre-diabetes and diabetes are 9.9% and 11.54% respectively.

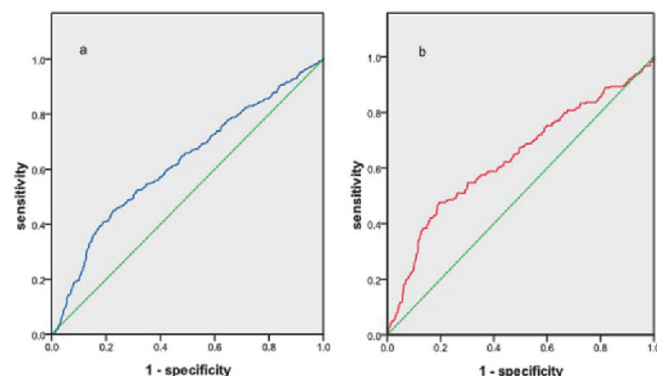


Fig. 1 Receiver operating characteristic (ROC) curves for LFC associated with an increased prevalence of pre-diabetes (a) and diabetes (b).

Supported by: National Key Technologies R&D Program

## PS 006 Lifestyle factors and diabetes

### 313

#### Comparison of current BMI and BMI histories to screen for undiagnosed diabetes in Japanese men: Toranomon Hospital Health Management Center Study

S. Yoshizawa<sup>1</sup>, Y. Heianza<sup>1,2</sup>, C. Horikawa<sup>1</sup>, S. Hara<sup>2</sup>, K. Saito<sup>1,2</sup>, S. Kodama<sup>1,2</sup>, S.D. Hsieh<sup>2</sup>, H. Tsuji<sup>2</sup>, N. Yamada<sup>1</sup>, Y. Arase<sup>2</sup>, H. Sone<sup>1,2</sup>

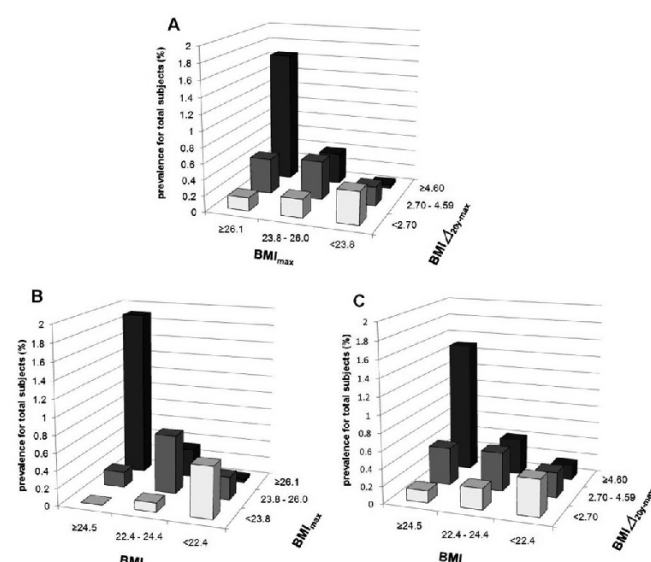
<sup>1</sup>Department of Endocrinology and Metabolism, University of Tsukuba Mito Medical Center, Mito, <sup>2</sup>Health Management Center, Toranomon Hospital, Tokyo, Japan.

**Background and aims:** Evidence has shown that about half of individuals with diabetes remain undiagnosed worldwide. Therefore, screening for undiagnosed diabetes is a major priority. Although the utility of BMI histories in determining risk of developing future diabetes has been studied, we know little about which obesity index would be most effective for screening of undiagnosed diabetes. We therefore aimed to directly compare current BMI and various BMI histories to identify a screening tool for undiagnosed diabetes in Japanese men.

**Materials and methods:** This cross-sectional study included 15143 Japanese men aged 30–75 years without a self-reported history of clinician-diagnosed diabetes. We estimated the probability of having undiagnosed diabetes (diabetes: fasting plasma glucose level of  $\geq 7.0$  mmol/L and/or HbA1c  $\geq 6.5\%$ ) for the following 6 obesity indices: (1) current BMI, (2) BMI at age 20 years ( $BMI_{20y}$ ), (3) lifetime maximum BMI ( $BMI_{max}$ ), (4) BMI change since age 20 years to current BMI ( $BMI\Delta_{20y-cur}$ ), (5) BMI change since age 20 years to maximum BMI ( $BMI\Delta_{20y-max}$ ), and (6) BMI change since maximum to current BMI ( $BMI\Delta_{max-cur}$ ). Logistic regression analysis was performed to investigate odds ratio (ORs) for undiagnosed diabetes using these indices as continuous or categorical variables.

**Results:** Prevalence of undiagnosed diabetes was 4.1% ( $n = 617/15143$ ) among the total subject population. Among the 6 obesity indices,  $BMI_{max}$  (1-SD increment) was most strongly associated with undiagnosed diabetes with an OR of 1.56 [95% CI: 1.45–1.68] after adjustment for age and lifestyle factors. ORs for current BMI or  $BMI\Delta_{20y-max}$  were 1.46 (1.36–1.57) or 1.46 (1.35–1.57), respectively, for undiagnosed diabetes. These 3 BMI histories had a strong association with undiagnosed diabetes compared to the remaining indices (OR 1.19 [1.11–1.29] for  $BMI_{20y}$ , 1.30 [1.21–1.41] for  $BMI\Delta_{20y-cur}$ , and 1.22 [1.14–1.30] for  $BMI\Delta_{max-cur}$ ). When we investigated the combined effect of  $BMI_{max}$  and  $BMI\Delta_{20y-max}$  in identifying undiagnosed diabetes, those in the top tertiles of both  $BMI_{max}$  and  $BMI\Delta_{20y-max}$  had a markedly higher prevalence of undiagnosed diabetes than other individuals (Figure. Panel A). Among obese individuals with current BMI  $\geq 24.5$  kg/m<sup>2</sup> (Panels B, C), having a history of either the top tertile of  $BMI_{max}$  or  $BMI\Delta_{20y-max}$  escalated the likelihood of undiagnosed diabetes. Conversely, in non-obese individuals with a current BMI of  $< 24.5$  kg/m<sup>2</sup>, we observed a substantially low prevalence of undiagnosed diabetes.

**Conclusion:** Our results of direct comparisons of 6 BMI histories showed that both  $BMI_{max}$  and  $BMI\Delta_{20y-max}$  were the most strongly associated with current undiagnosed diabetes. This combination would be a useful tool to screen for diabetic individuals who were unaware of their condition.



**Figure:** Prevalence of undiagnosed diabetes according to combined effect of BMI histories

Supported by: The Ministry of Health, Labour and Welfare, Japan

## 314

### Defining overweight and obesity in 18550 paediatric patients with type 1 diabetes from Germany and Austria - which reference to use?

M. Flechtner-Mors<sup>1</sup>, E.E. Fröhlich-Reiterer<sup>2</sup>, T.M. Kapellen<sup>3</sup>, T. Meissner<sup>4</sup>, J. Rosenbauer<sup>5</sup>, K.O. Schwab<sup>6</sup>, R. Stachow<sup>7</sup>, R.W. Holl<sup>1</sup>, the DPV Initiative and the BMBF competence networks diabetes and obesity;

<sup>1</sup>University Ulm, Germany, <sup>2</sup>Medical University Graz, Austria, <sup>3</sup>University Leipzig, <sup>4</sup>University Düsseldorf, <sup>5</sup>DDZ University Düsseldorf, <sup>6</sup>University Hospital Freiburg, <sup>7</sup>Specialised Clinic for Children and Adolescents, Westerland/Sylt, Germany.

**Background and aims:** Modern intensive insulin therapy / CSII are associated with weight gain, increasing the future cardiovascular risk of the patients. However, the diagnosis of overweight or obesity during childhood depends on the choice of a reference population. Therefore, we compared the rates of overweight and obesity in children and adolescents with type 1 diabetes (T1D) according to four body mass index (BMI) references. In addition, time trend during recent years were examined.

**Material and methods:** The Diabetes and Quality Management System (DPV) is a standardized, longitudinal documentation for patients with diabetes. Over a twelve year period from 2000 (n=10194) to 2011 (n=18550) height and weight of children and adolescents aged between 2 and 18 years, were calculated. Prevalence estimates of overweight and obesity were determined. Three BMI categories were used: non-overweight <90<sup>th</sup> percentile, overweight ≥90<sup>th</sup> - <97<sup>th</sup> percentile and obesity ≥97<sup>th</sup> percentile based on growth curves for age and sex generated by the World Health Organization (WHO), the International Obesity Task Force (IOTF), the German Working Group on Obesity in Childhood and Adolescents (AGA) and the German Health Interview and Examination Survey of Children and Adolescents (KiGGS). Prevalence estimates were adjusted for diabetes duration, age and sex. Statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, USA).

**Results:** In children and adolescents with T1D, the prevalence of overweight is highest according to IOTF, followed by WHO, AGA and KiGGS. In contrast, the prevalence of obesity according to WHO is higher compared to AGA, KiGGS and IOTF. More girls are overweight and obese than boys (exception: obesity WHO)(Table, percentages, f=female, m=male). Irrespective of references, the percentage of overweight and obese children and adolescents with T1D increased between 2000 and 2011 (p<0.0001).

BMI category	overweight ≥90 <sup>th</sup> - 97 <sup>th</sup> P.			obese ≥97 <sup>th</sup> P.								
year	2000			2011			2000			2011		
	all	f	m	all	f	m	all	f	m	all	f	m
WHO (%)	13.2	13.8	12.7	14.8	15.5	14.2	8.1	7.7	8.5	10.5	9.4	11.5
AGA (%)	11.0	11.8	10.2	12.5	13.5	11.6	4.6	5.9	3.4	6.9	7.7	6.1
KiGGS (%)	7.8	8.5	7.0	9.5	10.1	8.9	2.1	2.5	1.6	3.1	3.2	3.1
IOTF (%)	19.3	21.4	17.2	21.8	23.8	19.9	3.1	3.8	2.5	5.0	5.2	4.9

**Conclusion:** Different reference populations influence the classification of weight categories. The proportion of overweight and obesity in children and adolescents with type 1 diabetes is rising. More girls than boys are overweight and obese according to AGA, KiGGS and IOTF.

Clinical Trial Registration Number: 01GI0859

Supported by German Federal Ministry of Education and Research

## 315

### Anthropometric indices of obesity in relation to cardiometabolic disorders in Chinese adults

M. Xu<sup>1</sup>, L. Zhang<sup>1,2</sup>, N. Wang<sup>1</sup>, J. Li<sup>1</sup>, Y. Dong<sup>1,2</sup>;

<sup>1</sup>Qingdao Endocrine & Diabetes Hospital, Qingdao, <sup>2</sup>Weifang Medical University, Weifang, China.

**Background and aims:** Anthropometric indicators such as body mass index (BMI), waist circumference (WC), waist to hip ratio (WHR), and waist to stature ratio (WSR) are used to relate cardiometabolic risk. This study was aimed to identify the optimal anthropometric index in relation to diabetes, hypertension, and dyslipidemia.

**Materials and methods:** We enrolled 15268 Chinese adults aged 18-74 years (M/F: 7018/8250) who participated in a population-based cross-sectional survey conducted in Qingdao, China from 2001-2002. Information on demographic details as well as body weight, height, WC and HC, blood pressure (BP), fasting plasma glucose (FPG), 2-h plasma glucose (2hPG), blood lipid profile were measured. Receiver operating characteristic (ROC) curve was plotted and the optimal cutoff point was identified. Diagnostic accuracy was assessed by the area under the curve (AUC). Multiple logistic regression analysis was used to examine the independent relationship between each anthropometric index and the risk of having cardiometabolic disorders.

**Results:** BMI, WSR, WHR and WC were positively associated with systolic BP (SBP), diastolic BP (DBP), FPG, 2hPG, triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) in both men and women (P<0.01), but negatively related to high-density lipoprotein cholesterol (HDL-C) (P<0.01). WSR was found to have the largest AUC for diabetes (0.64, 95%CI: 0.62-0.65), hypertension (0.68, 95%CI: 0.67-0.70), and dyslipidemia (0.58, 95%CI: 0.55-0.59) in both genders, followed by WC, BMI, and WHR. The optimal cut-off points of WSR for diabetes, hypertension, and dyslipidemia were 0.52, 0.51, and 0.53, respectively. The odds ratio (OR) of WSR were 1.87 (95%CI: 1.57-2.24) in men and 1.74 (95%CI: 1.54-1.82) in women for having diabetes, 1.80 (95%CI: 1.52-2.11) and 2.43 (95%CI: 2.09-2.82) for hypertension, and 1.40 (95%CI: 1.19-1.65) and 1.31 (95%CI: 1.07-1.61) for dyslipidemia, respectively.

**Conclusion:** WSR might be the optimal anthropometric index in assessing cardiometabolic risk in Chinese adults.

Supported by: QHB

## 316

### A habitually higher dietary glycaemic index during puberty is prospectively related to increased risk markers of type 2 diabetes in young adulthood

J. Goletzke<sup>1</sup>, C. Herder<sup>2</sup>, G. Joslowski<sup>1</sup>, K. Bolzenius<sup>1</sup>, T. Remer<sup>1</sup>, S.A. Wudy<sup>3</sup>, W. Rathmann<sup>2</sup>, M. Roden<sup>2,4</sup>, A.E. Buyken<sup>1</sup>;

<sup>1</sup>Research Institute of Child Nutrition, Dortmund, <sup>2</sup>German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, <sup>3</sup>Laboratory for Translational Hormone Analytics in Pediatric Endocrinology, Center of Child and Adolescent Medicine, Justus-Liebig-University, Giessen, <sup>4</sup>Department of Metabolic Diseases, University Hospital Düsseldorf, Heinrich-Heine University, Düsseldorf, Germany.

**Background and aims:** Carbohydrate nutrition during periods of physiological insulin resistance such as puberty may be of particular relevance for future

risk of type 2 diabetes. The aim of this study was to investigate whether the amount (% energy) or the quality (dietary glycemic index, GI; glycemic load, GL; added sugar and fiber intake) of carbohydrates during puberty relates to markers of insulin resistance and fatty liver (steatosis) in young adulthood.

**Materials and methods:** The analysis was based on 208 participants (115 girls and 93 boys) from the DONALD study with at least two 3-day weighed dietary records during puberty (girls: 9–14 years, boys: 10–15 years) and blood samples in young adulthood (18–35 years). Multivariate linear regression models were used to analyze the associations between carbohydrate nutrition and homeostasis model assessment (HOMA-IR) index ( $n=202$ ), the liver enzymes alanine-aminotransferase (ALT), and gamma-glutamyltransferase (GGT) as well as fasting serum triglycerides (TG).

**Results:** A higher dietary GI was prospectively related to greater values of HOMA-IR ( $p$  for trend = 0.03), ALT ( $p$  for trend = 0.03), and GGT ( $p$  for trend = 0.04). After adjustment for sex, age (adult), early life and parental socioeconomic factors as well as protein and fiber intake, predicted HOMA-IR values in energy-adjusted tertiles of GI were 2.41 (95% confidence intervals: 2.17, 2.68), 2.56 (2.31, 2.83), 2.74 (2.46, 3.05). No prospective associations were observed between GI and TG. In contrast to GI, neither the amount of carbohydrates nor GL, added sugar (total or from drinks) or fiber intake were related to any of the analyzed markers. Associations between carbohydrate nutrition and risk markers of type 2 diabetes did not differ by sex ( $p$  for interactions > 0.2).

**Conclusion:** These data indicate that a habitually higher dietary GI during puberty may adversely affect risk markers of type 2 diabetes in young adulthood. Preferred selection of low-GI carbohydrates during puberty could be useful during puberty.

Supported by: BMELV through BLE

## 317

### Lifestyle risk factors and preventable proportion of autoimmune diabetes in adults: 22 years follow-up of the HUNT study

S. Carlsson<sup>1</sup>, V. Grill<sup>2</sup>, K. Midtjell<sup>3</sup>, T. Andersson<sup>1</sup>, B. Rasouli<sup>1</sup>

<sup>1</sup>Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, <sup>2</sup>NTNU Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, <sup>3</sup>HUNT Research Centre, Norwegian University of Science and Technology, Levanger, Norway.

**Background and aims:** Although autoimmune diabetes in adults (AIDA) is prevalent, there is limited data on potential risk factors and the preventive potential of AIDA is unknown. Our aim was to provide a synthesis of available data on lifestyle factors linked to AIDA from the large population-based Nord-Trøndelag Health Study (HUNT), and estimate the proportion of preventable cases if these exposures could be eliminated.

**Materials and methods:** We used data from HUNT, in which adults aged  $\geq 20$  years old were investigated in three consecutive surveys during 1984–2008. Incident cases of diabetes were identified by self-report and subjects with onset age  $\geq 35$  years were classified as type 2 diabetes if they were anti-GAD negative ( $n=2244$ ) and as having autoimmune diabetes if they were anti-GAD positive ( $n=164$ ). Hazard ratios (HR) associated with lifestyle factors, adjusted for potential confounders were estimated by Cox proportion hazards regression models. Population attributable risks (PAR) was estimated for combinations of lifestyle factors, with the lowest risk level as the referent.

**Results:** In multivariate survival analysis, the following factors were associated with AIDA: overweight (HR=2.26, 95 % CI=1.31–3.88), physical inactivity (HR=3.51 95 % CI=1.39–8.88, smoking (HR=0.56 95 % CI=0.34–0.91), moderate alcohol consumption (HR= 0.36, 95 % CI=0.13–0.97), low psychological wellbeing (HR=1.65 95 % CI=0.98–2.79), and family history of diabetes (HR=3.19 95 % CI=2.15–4.74). Similar findings were seen for type 2 diabetes, except for smoking which was associated with an increased risk (HR1.39, 95 % CI 1.07–1.77). Subjects with BMI, physical activity level, psychosocial well-being, and alcohol use in the low risk group had HR for AIDA of 0.16, 95% CI=0.10–0.28 with PAR estimated at 72 % (0.6–93%) with low BMI as the most important protective factor. Corresponding estimates for type 2 diabetes were 0.26, 95% CI=0.6–1.05, PAR, 82% (70–89%).

**Conclusion:** Our findings indicate that the vast majority of AIDA cases are preventable by modification of common lifestyle factors such as weight reduction and physical activity, and that AIDA is similar to type 2 diabetes in this respect.

**Table:** Risk of incident of type 2 and autoimmune diabetes and population attributable risk according to low-risk lifestyle factors.

No. of low-risk lifestyle factors†	No. Cases Exposed/non-exposed (n/n)	Participants in low risk group/all other not in low risk group (n%)	HR* (95% CI)	Population attributable risk ‡ (95% CI), %
<b>Type 2 diabetes</b>				
2 factors in low-risk category (physically active, and alcohol use $\leq 1$ time/ during 2 weeks)	255/1370	16/90 (24.32%) 51/883 (25.88)	0.86 (0.75–0.99)	11 (3–20)
3 factors in low-risk category (2 above plus no heavy smoking)	212/1213	15/151 (23.16%) 53/222 (77.81)	0.87 (0.70–0.96)	14 (3–24)
4 factors in low-risk category (3 above plus without sleep disturbances and low psychosocial well-being)	136/1286	10/771 (15.75%) 57/602 (94.25)	0.78 (0.55–0.94)	19 (5–31)
5 factors in low-risk category (4 above plus BMI $< 25$ )	14/1411	5/783 (8.46%) 62/990 (91.54)	0.16 (0.10–0.28)	82 (70–89)
<b>Autoimmune diabetes in adults</b>				
2 factors in low-risk category (physically active, and alcohol use $\leq 1$ time/ during 2 weeks)	17/98	16/966 (23.93%) 5/969 (26.06)	0.69 (0.40–1.19)	25 (0.70–53)
3 factors in low-risk category (2 above plus without sleep disturbances and low psychosocial well-being)	13/102	11/853 (16.71%) 5/903 (33.29)	0.75 (0.41–1.38)	22 (0.7–55)
4 factors in low-risk category (3 above plus BMI $< 25$ )	2/113	5/431 (8.80%) 64/691 (91.20)	0.26 (0.06–1.05)	77 (0.6–93)

\*Adjusted for age [years], sex (male vs. female) and family history of diabetes [presence of any FHD vs. Absence of FHD], and each lifestyle risk factors not already included in the model. Compared with all other participants not in this low risk group

†The population attributable risk is the percentage of cases in the population that would theoretically not have occurred if all participants had been in low-risk category for these factors.

‡Low-risk lifestyle factors included physically activity (very high active, and high active), heavy smoking (only for type 2 diabetes), alcohol use ( $\leq 1$  time during 2 weeks), BMI ( $< 25 \text{ kg/m}^2$ ), without sleep disturbance and low psychosocial well-being

Supported by: Nord-Trøndelag County Council, Norwegian Institute of Public Health, Glaxo

## 318

### A low level of cardiorespiratory fitness during college-age is a precursor of adult-onset diabetes mellitus

Y. Someya<sup>1</sup>, S. Kawai<sup>1</sup>, N. Saga<sup>1</sup>, Y. Kohmura<sup>1</sup>, K. Aoki<sup>1</sup>, E. Marui<sup>2</sup>, H. Daida<sup>3</sup>

<sup>1</sup>School of Health and Sports Science, Juntendo University, Chiba,

<sup>2</sup>Department of Public Health, Juntendo University, Tokyo, <sup>3</sup>Department of Cardiovascular Medicine, Juntendo University, Tokyo, Japan.

**Background and aims:** In recent years, the occurrence of diabetes has increased greatly in Asian countries. There has also been a rapid growth in diabetes in Japan, which is now one of the nations most affected by the worldwide diabetes epidemic. Type 1 diabetes is rare in Japan, with type 2 diabetes predominating in both adults and children. In European-derived populations, overweight and obesity are established causes of diabetes. However, many Japanese diabetics are not obese. An association between physical fitness during college-age and adult-onset diabetes mellitus has not been demonstrated. This study therefore examined the relationship between physical fitness in college-age students and development of adult-onset diabetes in Japanese men.

**Materials and methods:** From 2009 to 2011, we mailed follow-up questionnaires to former students of Juntendo University, with questions regarding the occurrence of doctor-diagnosed adult-onset diabetes mellitus and their lifestyle. The incidence rate of diabetes mellitus during the follow-up period was compared with stored data of a physical fitness test carried out at college (Japanese national physical fitness test: 50 meter dash, running broad jump, pull-ups, hand ball throw, and 1,500 meter endurance run).

**Results:** The responders who had graduated from Juntendo University between 1975 and 1991 included 577 men, 23 (4%) of whom had developed adult-onset diabetes. Mean age was  $20 \pm 2$  years at the college physical fitness test and  $48 \pm 4$  years at the time of the follow-up questionnaire. The subjects with diabetes had increased weight and waistline at the time of the questionnaire (diabetics vs. non-diabetic former students: weight  $77.9 \pm 13.4$  vs.  $72.0 \pm 9.6$  kg, waistline  $89.6 \pm 9.4$  vs.  $83.6 \pm 6.7$  cm), with their gross physical fitness test score and 1,500 meter endurance run (cardiorespiratory fitness level) at college-age being lower than in the non-diabetic former students (diabetics vs. non-diabetic former students: gross physical fitness test score  $63.9 \pm 12.8$  vs.  $57.0 \pm 12.8$  points, 1,500 meter endurance run time  $325.8 \pm 32.5$  vs.  $345.3 \pm 34.8$  seconds). A Cox proportional hazards model across quartiles of cardio-respiratory fitness level (lowest to highest), age, and weight gain since the college physical fitness test until the time of the questionnaire showed the adjusted relative risks for developing adult-onset diabetes mellitus were 1.00 (reference), 0.54 (95% CI: 0.20–1.49), 0.04 (0.05–0.95), and 0.03 (0.07–0.90), respectively ( $p < 0.05$  for trend).

**Conclusion:** These results indicate that a low cardiorespiratory fitness level during college-age may be an important risk factor for the development of adult-onset diabetes in Japanese men.



## 319

**Leisure-time physical activity is a significant predictor for total mortality and stroke among Japanese patients with type 2 diabetes: the Japan Diabetes Complications Study**H. Sone<sup>1</sup>, S. Tanaka<sup>2</sup>, S. Tanaka<sup>2</sup>, S. Suzuki<sup>3</sup>, H. Seino<sup>3</sup>, A. Sato<sup>4</sup>, A. Araki<sup>5</sup>, S. Ishibashi<sup>6</sup>, Y. Ohashi<sup>7</sup>, Y. Akanuma<sup>8</sup>, N. Yamada<sup>1</sup>;<sup>1</sup>University of Tsukuba, Ibaraki, <sup>2</sup>Kyoto University, <sup>3</sup>Ohta General Hospital, Fukushima, <sup>4</sup>Tokyo Women's Medical University, <sup>5</sup>Tokyo Metropolitan Geriatric Hospital, <sup>6</sup>Jichi Medical College, Tochigi, <sup>7</sup>University of Tokyo,<sup>8</sup>Institute for Adult Diseases Asahi Life Foundation, Tokyo, Japan.

**Background and aims:** Although physical activity is reportedly associated with mortality and cardiovascular disease among patients with diabetes, only one study has determined both mortality and cardiovascular events simultaneously, so it is still unclear how physical activity exerts its effect on reducing mortality among diabetic patients. Furthermore, no study has examined the relationship between cardiovascular disease and physical activity among Asian diabetic patients despite the fact that Asian patients account for more than 60% of the world's diabetic population. The aim of this study was to clarify the association between leisure-time physical activity (LTPA), which accounts for an important part of exercise therapy for diabetic subjects, and cardiovascular events and total mortality in a nationwide cohort of Japanese diabetic patients.

**Subjects and methods:** In total, 1702 eligible patients with type 2 diabetes (mean age, 58.5 y; 47% women) from 59 institutes were followed for a median period of 8.05 years. A comprehensive lifestyle survey including LTPA and occupation was carried out using standardized questionnaires. Outcomes were the occurrence of coronary heart disease (CHD) or stroke, and total mortality. The adjusted hazard ratio (HR) and 95% confidence interval (CI) was calculated by Cox regression analysis.

**Results:** A significant reduction in HR among patients in the top ( $\geq 15.4$  METs-hr/week) vs. the bottom ( $< 3.7$  METs-hr/week) tertile of LTPA, adjusted by age, sex and diabetes duration, was observed for stroke (HR 0.55, 95%CI 0.32–0.94) and total mortality (0.49, 0.26–0.91) but not in CHD (0.77, 0.48–1.25). The HR for stroke became nonsignificant after adjustment for clinical variables comprising typical cardiovascular risk factors. The significant reduction in total mortality due to LTPA was independent of lifestyle or clinical variables, and was not, at least mainly, attributed to reduced cardiovascular disease.

**Conclusion:** Among Japanese subjects with type 2 diabetes, an LTPA of more than 15.4 METs-hr/week was associated with a significantly lower risk of stroke through ameliorating combinations of cardiovascular risk factors. It was also associated with a significant reduction in total mortality but was independent of cardiovascular risk factors or events. These findings, which imply differences from Western diabetic populations, should be considered for the clinical management of East Asians with diabetes.

## 320

**Smoking is associated with reduced risk of autoimmune diabetes in adults contrasting with increased risk of type 2 diabetes: a 22-year follow-up of the HUNT study**B. Rasouli<sup>1</sup>, A. Ahlbom<sup>1</sup>, T. Andersson<sup>1</sup>, V. Grill<sup>2</sup>, K. Midtjell<sup>3</sup>, S. Carlsson<sup>1</sup>;<sup>1</sup>Department of Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, <sup>2</sup>NTNU Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Levanger, Norway, <sup>3</sup>HUNT Research Centre, Department of Community Medicine and General Practice, Norwegian University of Science and Technology, Levanger, Norway.

**Background and aims:** Smoking has been associated with an increased risk of type 2 diabetes but the influence on autoimmune diabetes is not clear. We aimed to investigate the association between smoking habits and risks of autoimmune diabetes in adults and type 2 diabetes.

**Materials and methods:** For this prospective cohort study we used data from the three surveys of the Helseundersøkelser in Nord Trøndelag (HUNT) study, spanning 1984 to 2008 and including a cohort of 90,819 participants aged  $\geq 20$  years old. Eligible population for the present study recruited from subjects who were free of diabetes at baseline with complete information regarding smoking habits, age and sex, they followed up for incidence of type 2 diabetes or autoimmune diabetes. Incident cases of diabetes were identified by questionnaire and classified as type 2 diabetes (n=1860) and autoimmune diabetes (n=140) based on anti-GAD and age at onset of diabetes. Hazard

ratios (HR) adjusted for age, sex, BMI and education were estimated by Cox proportion hazards regression models.

**Results:** The risk of autoimmune diabetes was reduced by 48% (HR=0.52, 95% confidence intervals, CI=0.30–0.89) in current smokers and 58% in heavy smokers (HR for  $\geq 20$  cigarettes/day=0.42, 95% CI=0.18–0.98). The reduced risk was associated with number of pack years (HR for  $\geq 13$  pack-years= 0.43, 95% CI 0.23–0.82). Heavy smoking was associated with lower levels of anti-GAD (p=0.001) and higher levels of C-peptide (964.22 vs. 886.15 pmol/l, p=0.03). In contrast, smoking was associated with an increased risk of type 2 diabetes, a risk which was however restricted to overweight men (HR for current smoking= 1.33, 95% CI=1.10–1.61; HR for  $\geq 20$  cigarettes/day=1.70, 95% CI=1.38–2.10; and HR for  $\geq 13$  pack-years=1.57, 95% CI=1.31–1.90). Attributable proportion of type 2 diabetes due to an interaction between overweight and heavy smoking was estimated at 0.40 (95% CI=0.23–0.57).

**Conclusion:** Smoking was associated with a reduced risk of autoimmune diabetes which appears linked to immunosuppression and anti-inflammatory effects of nicotine. In contrast, smoking increases risk of type 2 diabetes risk in overweight men, and overweight and smoking likely interact to induce type 2 diabetes.

HR of autoimmune diabetes in adults and type 2 diabetes in relation to smoking, results from HUNT study (1984–2008)

	Autoimmune diabetes in adults			Type 2 diabetes					
				BMI <25 (kg m <sup>-2</sup> )			BMI $\geq 25$ (kg m <sup>-2</sup> )		
	Person-year	No. cases	HR <sup>b</sup> (95% CI)	No. cases	HR <sup>c</sup> (95% CI)	Women	No. cases	HR <sup>c</sup> (95% CI)	Women
<b>Smoking</b>									
Never	445,658	75	Reference	115	Reference	Reference	723	Reference	Reference
			1.10		0.83	0.50		1.31	0.87
Former	229,225	40	(0.72–1.68)	46	(0.52–1.33)	(0.25–1.02)	490	(1.10–1.57)	(0.71–1.08)
			0.52		0.60	0.60		1.33	0.98
Current	293,758	25	(0.30–0.89)	68	(0.38–0.96)	(0.36–1.00)	418	(1.10–1.61)	(0.81–1.20)
<b>Cigarettes per day (former and current smokers)</b>									
Never	445,658	75	Reference	115	Reference	Reference	723	Reference	Reference
Light smokers (<20)	396,335	52	0.86 (0.59–1.26)	91	0.72 (0.47–1.11)	0.64 (0.41–1.02)	621	1.27 (1.07–1.52)	0.93 (0.78–1.10)
Heavy smokers ( $\geq 20$ )	68,458	7	0.42 (0.18–0.98)	17	1.10 (0.60–1.99)	-	194	1.70 (1.38–2.10)	1.16 (0.78–1.73)
<b>Cumulative quantity of active smoking (pack-years)</b>									
Never	444,399	75	Reference	115	Reference	Reference	722	Reference	Reference
<6	109,445	13	1.19 (0.65–2.18)	18	0.92 (0.44–1.89)	0.72 (0.33–1.53)	126	0.95 (0.67–1.32)	1.04 (0.80–1.35)
6–12	120,439	19	1.11 (0.64–1.93)	18	0.64 (0.33–1.24)	0.50 (0.23–1.12)	170	1.26 (0.99–1.60)	0.84 (0.63–1.11)
$\geq 13$	14,125	17	0.43 (0.23–0.82)	50	0.73 (0.45–1.19)	0.53 (0.25–1.13)	384	1.57 (1.31–1.90)	0.99 (0.77–1.27)
<sup>a</sup> Numbers of cases are before including in multivariate analysis models									
<sup>b</sup> HR adjusted for age, sex, BMI, education and physical activity									
<sup>c</sup> HR adjusted for age, sex, education and physical activity									

Supported by: HUNT research center (Faculty of Medicine of NTNU), NTFK, FHI, NTNU

## PS 007 Environmental factors in type 1 diabetes

321

### Entred-Ado study: health, education and risk behaviours of adolescents with diabetes

I. Milovanovic<sup>1</sup>, M. Chantry<sup>2</sup>, I. Romon<sup>3</sup>, C. Druet<sup>3</sup>, A. Fagot-Campagna<sup>3</sup>, C. Levy-Marchal<sup>1</sup>;

<sup>1</sup>INSERM-CIE05, Paris, <sup>2</sup>Caisse Nationale d'Assurance Maladie des Travailleurs Salariés, Paris, <sup>3</sup>Institut de Veille Sanitaire, Saint-Maurice, France.

**Background and aims:** The adolescence is a critical moment for diabetic patients representing a period of potential low adherence and poor metabolic control. The aim of present study was to describe the health status, treatment and quality of care of adolescents with diabetes in France and to study educational level, risk-taking behaviors, and quality of life of those young patients.

**Materials and methods:** A 7 % random selection from all the diabetic patients, aged 11–18 years, covered by the French Social Security identified 554 eligible diabetic adolescents. The rate of participation was 51 %. A specifically developed telephone questionnaire was used covering the following topics: health and diabetes, education, risk behaviors and quality of life. These 283 adolescents with diabetes were compared to 115 classmates.

**Results:** Of all responding adolescents 98% had type 1 diabetes. Adolescents younger or older than 16.5 years ( $n = 139$  vs.  $n = 144$ ), were not statistically different in terms of treatment (2 injections: 7% vs. 9%; multi-injections: 67% vs. 75%; pump: 26% vs. 16%;  $p=0.4$ ), HbA1c ( $8.2 \pm 1.4$  vs.  $8.7 \pm 1.9$ ;  $p=0.08$ ), frequency of DKA (6% vs. 11%;  $p=0.1$ ; median = 1/yr) and severe hypoglycemia (9% vs. 4%;  $p=0.1$ ; median = 1/yr). Glycemic control was not influenced by the insulin regimen (2 injections  $8.3 \pm 0.5\%$  HbA1c; multi-injections:  $8.6 \pm 0.1\%$  HbA1c; pump:  $8.3 \pm 0.3\%$  HbA1c;  $p=0.5$ ) whereas HBGM was significantly different between the regimens. 90 % of the adolescents were under hospital care (4 visits /yr). Compared to 115 control adolescents, the rate of school grade repetition was higher (43% vs. 30%;  $p=0.03$ ) and the average school note was lower at adolescents with diabetes (12–20/20: 53% vs. 66%;  $p=0.03$ ). Moreover the rate of absenteeism was higher in the diabetic group with 56% of diabetic adolescents claiming that they were absent from school from time to time and more than 10 % feeling that they were frequently absent from school, comparing with 32% and 4% respectively among their healthy classmates ( $p<0.01$ ). There was no significant difference between adolescents with diabetes and their controls in percentage of adolescents having experienced at least once smoking (39% vs. 41%;  $p=0.8$ ), alcohol (52% vs. 60%;  $p=0.2$ ) or cannabis (14% vs. 17%;  $p=0.4$ ), but these risk behaviors were more regular in the control group. The quality of life of diabetic adolescents assessed by PedsQL questionnaire did not differ in health and emotions from controls, while the study score was lower ( $67 \pm 19$  vs.  $72 \pm 16$ ;  $p=0.01$ ) and the relational score higher ( $87 \pm 13$  vs.  $83 \pm 14$ ;  $p=0.01$ ).

**Conclusion:** This is the first French study in the general population being focused on adolescents with diabetes. Glycemic control is at similar level as in other large European studies, but above the recommendations. A modest but significant impact of diabetes on school performances was identified, not often pointed in the studies in adolescents.

322

### Are disordered eating behaviours more frequent in children and adolescents with type 1 diabetes? A comparison with a population-based sample from Germany

C. Bächle<sup>1</sup>, K. Castillo<sup>1</sup>, K. Straßburger<sup>1</sup>, A. Stahl<sup>1</sup>, T. Meissner<sup>2</sup>, R.W. Holl<sup>3</sup>, G. Giani<sup>1</sup>, J. Rosenbauer<sup>1</sup>, in cooperation with the German Pediatric Surveillance Unit (ESPED), the DPV-Science Initiative, and the Competence Network Diabetes mellitus;

<sup>1</sup>Institute of Biometrics and Epidemiology, German Diabetes Center, Düsseldorf, <sup>2</sup>University Children's Hospital Düsseldorf, <sup>3</sup>Institute of Epidemiology and Medical Biometry, University of Ulm, Germany.

**Background and aims:** Despite modern therapeutic regimens youths with type 1 diabetes (T1DM) may be at increased risk of mental and behavioural disorders including eating disorders. The aim of this study was to compare the prevalence of disturbed eating behaviours in children and adolescents with T1DM and their peers from the general population.

**Materials and methods:** Data of 629 T1DM patients from a population-based, nationwide survey (54.1% male, age 15.3 (SD 1.7) years, 48.7 (SD 0.5) % continuous subcutaneous insulin infusion) with early-onset diabetes (0–4 years) and at least 10 years diabetes duration (12.5 (SD 1.6) years) were compared with 6813 participants from the representative German KiGGS study (51.3% male, age 14.6 (SD 2.0) years). The SCOFF questionnaire was applied as screening instrument to identify subjects with suspicious eating behaviours. The gender-stratified comparison between the two groups was conducted by regression analyses adjusting for socio-demographic covariates. A sensitivity analysis was accomplished excluding SCOFF question 5 (Would you say food dominates your life?).

**Results:** 31.2% (11.7%) of the female (male) T1DM patients and 28.9% (15.2%) of the female (male) subjects of the comparison group were SCOFF positive ( $p>0.05$ ). The risk of patients with early-onset, long-lasting T1DM for symptoms of eating disorders was 5% higher for female, but 7% lower for male patients, yet not significantly different from the comparison group (adjusted OR female: 1.05, 95%-CI: 0.78–1.42; male: 0.93, 95%-CI: 0.62–1.38). Excluding question 5, the adjusted OR dropped to 0.78 for female (95%-CI: 0.54–1.12) and 0.37 for male patients (95%-CI: 0.17–0.77) indicating a lower prevalence of abnormal scores in male patients with T1DM.

**Conclusion:** Today, children and adolescents with early-onset T1DM do not seem to be more frequently affected by disturbed eating behaviours than their peers. The indicated lower risk may be explained by increased awareness of treating physicians.

Supported by: Competence Network Diabetes mellitus funded by BMBF (01GI0802, 01GI0859)

323

### Epidemic of DKA in Colorado: An indicator of childhood poverty and poor access to care

A. Rewers, M. Rewers;

The Barbara Davis Center for Childhood Diabetes, University of Colorado, Aurora, USA.

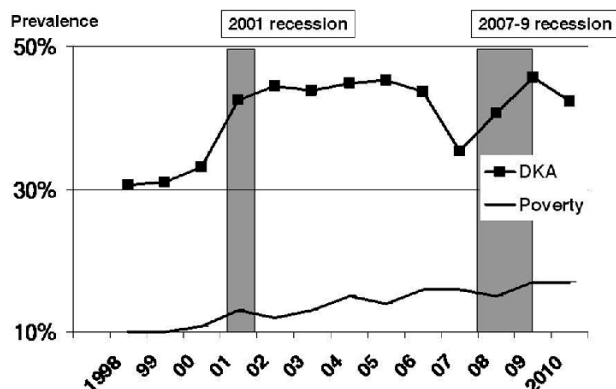
**Background and aims:** The frequency of diabetic ketoacidosis (DKA) at diagnosis is determined by community awareness of diabetes, access to medical care, and its quality. DKA rates have decreased or remained stable in most developed countries, during the past 20 years. This study examined the frequency of DKA at diagnosis and its predictors in Colorado children, during 1998–2010.

**Materials and methods:** Electronic patient record database of the Barbara Davis Center for Childhood Diabetes in Denver, ascertained 3257 children diagnosed with type 1 diabetes (T1D) in Colorado, during 1998–2010. Over 80% of children with diabetes in Colorado are seen at the Center within two years after diagnosis. T1D was present in 2953 (91%) of the children, using the American Diabetes Association criteria (the Center routinely tests all new patients for autoantibodies to insulin, GAD65 and IA-2, and more recently for ZnT8). Patients with type 2 diabetes ( $n=181$ , including 9 with DKA), diabetes secondary to other conditions, e.g., CF, steroid treatment ( $n=91$ , one with DKA) and those with monogenic or undefined type of diabetes ( $n=32$ , none with DKA) were excluded from further analyses. Medical records of all patients were reviewed for DKA at diagnosis (venous  $\text{pH}<7.3$  or bicarbonate  $<15$  mEq/l, in presence of hyperglycemia and ketosis). Data were insufficient to rule in/out DKA in 148 (5%) of T1D patients.

**Results:** The age-adjusted prevalence of Colorado children presenting in DKA has increased from 31.6% in 1998–2000 to an average of 44.3% during 2001–2010 ( $p=0.001$ ) [figure]. In multivariate logistic regression, younger age, low socioeconomic status and uninsurance were associated with DKA. On the population basis, this dramatic increase in severity of manifestation could be partially explained by a higher observed proportion of uninsured patients among children diagnosed more recently. The increase in DKA correlated ( $r=0.72$ ,  $p<0.02$ ) with the poverty rate in Colorado children (annual income below \$22,050 per family with two children, in 2010 dollars). The poverty rate has increased gradually from 10% (1998–2001) to 17% (2010) [figure].

**Conclusion:** The apparent epidemic of DKA in Colorado children over the past 10 years, despite raising community awareness of diabetes symptoms, points to problems with access to and quality of primary care. The rates of DKA in Colorado children, previously declining, are now twice as high as those in Europe. Our data support previous suggestions that rates of DKA at diagnosis correlate with poverty and income inequality.

## Prevalence of DKA at onset and poverty in Colorado children



## 324

## Age-period-cohort analysis of diabetes incidence time trends among 0 to 14 years-old children in Sardinia (Italy), 1989-2009

G. Bruno<sup>1</sup>, M. Maule<sup>2</sup>, F. Merletti<sup>2</sup>, A. Ledda<sup>3</sup>, V. Cau<sup>3</sup>, M. Songini<sup>3</sup>, Sardinian Group for Epidemiology of Type 1 Diabetes;

<sup>1</sup>Dept. Internal Medicine, Torino, <sup>2</sup>Cancer Epidemiology Unit, Torino,

<sup>3</sup>Diabetes Unit, Cagliari, Italy.

**Background and aims:** Sardinia is the second largest Mediterranean island and is characterized by one of the highest incidence rates of Type I diabetes in the world. Our aim was to analyze incidence time trends of childhood-onset Type I diabetes in Sardinia in period 1989-2009 using an age-period-cohort approach.

**Materials and methods:** Data on incident cases was obtained from the Sardinian Registry of Type I diabetes. Poisson regression models were used to estimate the effects of sex, age (5 three-year age groups: 0-2, ..., 12-14), calendar time (7 three-year periods: 1989-91, ..., 2007-09) and birth cohorts (11 six-year birth cohorts: 1974-79, ..., 2004-09). The models, hierarchically ordered, were compared by the likelihood ratio test.

**Results:** In 1989-2009, 2371 incident cases (1380 boys and 991 girls) were identified among children aged 0-14 years. Incidence rate was 44.85 cases per 100,000 person-years, (95% CI 43.08-46.69), with significantly lower risk in girls (38.70; 95% CI: 36.37-41.19) than in boys (50.63; 95% CI: 48.03-53.37). In regression analysis, after controlling for age, the rate ratio for girls with respect to boys was 0.76 (95% CI: 0.70-0.83). Incidence rate per 100,000 person-years increased from 35.78 in 1989-91 to 50.99 in 2007-2009. Controlling for age and sex, the annual increase was 2.12% (95% CI: 1.45-2.80; test for linear trend:  $p < 0.001$ ). Differences in time trends emerged between genders: among boys, annual percent changes were 4.4% ( $p < 0.001$ ), 2.4% ( $p = 0.034$ ), 0.8% ( $p = 0.423$ ), 1.1% ( $p = 0.214$ ) and 1.2% ( $p = 0.180$ ) in the age groups 0-2, 3-5, 6-8, 9-11, 12-14, respectively; among girls, the same figures were: 4.2% ( $p = 0.006$ ), 3.0% ( $p = 0.013$ ), 4.3% ( $p < 0.001$ ), 1.5% ( $p = 0.127$ ) and 1.2% ( $p = 0.314$ ). Results indicate that whereas for boys the increasing incidence time trend is limited to the youngest age group, it regards girls up to 8 years old. Age-period-cohort models were fitted separately to boys and girls. The best fitting model for boys included age and a linear time trend (drift), meaning that the variation over time had a linear component that could not be ascribed to either the calendar period or the birth cohort. The best fitting model for girls included age and both linear and non-linear effects of calendar period and birth cohort. The figure below shows, in both genders, age effects (expressed as incidence rates vs. age in the reference cohort (1992-97)) and cohort effects (relative risk with respect to the 1992-97 cohort); period effects were constrained to be 0 on average with 0 slope (rightmost and shorter curve).

**Conclusion:** Sardinia has very high and strongly increasing incidence time trends of childhood Type I diabetes. Incidence appears bimodal with age, with a first peak at approximately 3-4 years of age and a second stronger peak at 10-11 years of age. Incidence patterns differ between genders, with strongly non-linear increases among girls.

## 325

## Short chain fatty acids and neutrophils in the pathogenesis of type 1 diabetes

M.H. Harsunen<sup>1</sup>, S. Hummel<sup>1,2</sup>, A. Beyerlein<sup>1</sup>, G. Joslowski<sup>3</sup>, A. von Meyer<sup>4</sup>, M. Pflüger<sup>1,2</sup>, A.-G. Ziegler<sup>1,2</sup>;

<sup>1</sup>Helmholtz Zentrum München, Klinikum rechts der Isar, Technische

Universität München, Neuherberg, <sup>2</sup>Forscherguppe Diabetes e.V.,

Neuherberg, <sup>3</sup>Research Institute of Child Nutrition, Dortmund, <sup>4</sup>Institute of

Clinical Chemistry, Munich Municipal Hospital, Germany.

**Background and aims:** The short chain fatty acids acetate, propionate and butyrate are produced by bacterial fermentation of soluble dietary fiber (SDF) in the intestine and have functional immunomodulatory characteristics. They affect the production of proinflammatory mediators and chemotaxis through the GPR43 receptors, expressed on neutrophils. Recent studies suggest that early dietary factors and the microbial flora influence the development of islet autoimmunity and type 1 diabetes (T1D). We investigated the association between content of soluble fiber in the diet and occurrence of islet autoimmunity/T1D and whether individuals with T1D have altered neutrophil counts.

**Materials and methods:** The SDF intake was analysed using 448 3-day dietary records (after termination of breastfeeding) collected every 3 months from 134 children of the BABYDIET study, a dietary primary intervention study. During follow-up, 26 children developed islet autoantibodies and 7 T1D. We adjusted the content of SDF for total energy intake by calculating residuals from a linear model. The energy-adjusted SDF intake was compared in a case-control analysis between children with and without subsequent development of islet autoimmunity and T1D. Progression to autoimmunity and disease according to the energy-adjusted SDF intake was examined in a multivariable Cox regression model adjusted for age, gender, intervention group and family history of T1D. Neutrophil counts were analysed using hematological data from 107 individuals with new onset of T1D and 12 with islet autoimmunity, collected from participants of prospective diabetes studies, and 1196 age-matched anonymous controls, all individuals without laboratory signs of acute infection.

**Results:** The absolute neutrophil counts were significantly decreased in both T1D cases and individuals with islet autoimmunity compared with controls (median 3.11/nl and 2.33/nl vs 4.11/nl,  $p < 0.0001$ ). After adjustment for age and gender, the differences remained significant. The content of soluble fiber in the diet differed neither in the first year of life (1<sup>st</sup> measurement) nor in the second year of life (2<sup>nd</sup> measurement) between children who subsequently developed islet autoimmunity or T1D and respective controls (mean [SD] residuals 1<sup>st</sup> measurement: no seroconversion 0.16 [1.03], seroconversion -0.09 [1.10],  $p = 0.44$ ; mean [SD] residuals 2<sup>nd</sup> measurement: no seroconversion 0.48 [1.28], seroconversion -0.08 [1.64],  $p = 0.19$ ). Cox regression analyses revealed no significant association between soluble fiber intake and progression to seroconversion/T1D (HR of 1<sup>st</sup> measurement: 0.68 (95% CI: 0.24 - 1.97),  $p = 0.48$ ; HR of difference 1<sup>st</sup> vs 2<sup>nd</sup> measurement: 0.53 (0.17, 1.61),  $p = 0.26$ ).

**Conclusion:** We found no evidence of a reduced soluble fiber intake in the diet as a potential cause of disturbed immune regulation. However, our results suggest for the first time that dysfunction of neutrophils might be involved in the pathogenesis of T1D.

Clinical Trial Registration Number: NCT01115621

Supported by: JDRE, DFG, Stiftung "Das Zuckerkranken Kind", KKNDM

## 326

## Maternal fatty acid composition of diet during lactation and risk of preclinical and clinical type 1 diabetes

S. Niinistö<sup>1</sup>, H.-M. Takkinen<sup>2</sup>, L. Uusitalo<sup>1</sup>, J. Nevalainen<sup>3</sup>, M. Kenward<sup>4</sup>, M. Lumia<sup>1</sup>, O. Simell<sup>3</sup>, R. Veijola<sup>5</sup>, J. Ilonen<sup>3</sup>, M. Knip<sup>6</sup>, S.M. Virtanen<sup>1</sup>;

<sup>1</sup>Lifestyle and Participation, National Institute for Health and Welfare,

Helsinki, Finland, <sup>2</sup>University of Tampere, Finland, <sup>3</sup>University of Turku,

Finland, <sup>4</sup>London School of Hygiene & Tropical Medicine, UK, <sup>5</sup>University

of Oulu, Finland, <sup>6</sup>University of Helsinki and Helsinki University Central

Hospital, Finland.

**Background and aims:** We examined maternal dietary fatty acid intake during lactation and whether maternal intake is associated with the risk of pre-clinical and clinical type 1 diabetes in the offspring.

**Materials and methods:** The subjects comprised a cohort of 2939 mother-child pairs from the prospective Type 1 Diabetes Prediction and Prevention (DIPP) study. Composition of maternal diet during the 3rd month of lactation was assessed by a validated food frequency questionnaire. Among the



children with HLA-conferred susceptibility to type 1 diabetes, 143 developed preclinical type 1 diabetes (repeated positivity for islet cell autoantibodies in combination with one or more of the autoantibodies against insulin, glutamic acid decarboxylase or IA-2 protein), and 64 progressed to clinical diabetes. Average follow-up time was 4.1 years [range 0.5–9.7 years]. Piecewise linear log-hazard survival model and Cox proportional hazards regression were used for statistical analyses.

**Results:** Maternal intake of fatty acids during lactation was not associated with the risk of type 1 diabetes in the offspring. Maternal use of oils during lactation was associated with increased risk of preclinical type 1 diabetes (HR 1.22 [95% CI 1.04–1.44]). Low consumption of fresh milk during lactation was associated with higher risk for preclinical type 1 diabetes but only during the first 3 years of life (lowest quarter vs. intermediate half HR 2.04 [95% CI 1.16–3.58]).

**Conclusion:** The observed weak associations suggest that maternal consumption of oils and fresh milk during lactation might have some influence on the development of preclinical type 1 diabetes in the offspring with HLA-conferred susceptibility to type 1 diabetes.

Clinical Trial Registration Number: NCT00223613

Supported by: EFSD/Novo Nordisk grant

## 327

### Effect of high fat diet on serum endotoxin accumulation

M.I.S. Lassenius<sup>1</sup>, V.-P. Mäkinen<sup>1</sup>, M. Jauhiainen<sup>2</sup>, D. Gordin<sup>1</sup>, P. Pussinen<sup>3</sup>, M.-R. Taskinen<sup>4</sup>, J. Kirveskari<sup>5</sup>, C. Forsblom<sup>1</sup>, P.-H. Groop<sup>1</sup>, M. Lehto<sup>1</sup>, FinnDiane Study group;

<sup>1</sup>Folkhälsan Research Inst, Helsinki, <sup>2</sup>National Inst of Health and Welfare, Helsinki, <sup>3</sup>Inst of Dentistry, Univ of Helsinki, <sup>4</sup>Dep of Medicine, Divi of Cardiology, HUCH, <sup>5</sup>Dep of Bacteriology, HUSLAB, Helsinki, Finland.

**Background and aims:** We have recently shown that high levels of endotoxins/lipopolysaccharides (LPS) are associated with features of the metabolic syndrome and the development of kidney disease in patients with type 1 diabetes (T1D). In mammals, an energy rich diet promotes the translocation of gut-derived endotoxins together with chylomicrons to the circulation. Our aim is to investigate whether consecutive high fat meals in healthy controls and T1D patients without nephropathy lead to LPS accumulation.

**Materials and methods:** Participants (controls n=39; T1D n=37) were given three consecutive high fat meals at 8.00, 12.00, and 16.00 (total of 2600kcal). Blood was drawn at fasting and every two hours. Serum LPS activity was measured with the LAL assay (Hycult Biotech). Lipid parameters were determined by standard laboratory assays (HUSLAB, Helsinki) and NMR spectroscopy (NMR metabolomics Laboratory, Kuopio).

**Results:** Consecutive high fat meals had only a modest effect on LPS accumulation. Controls had a higher LPS incremental area (iA) of the initial meal response (8.00–12.00) compared to T1D patients [median 25th–75th quartile: 0.19 (–.31–0.98) vs. –.30 (–.81–0.32); p 0.025]. Fasting LPS and LPS area under curve (AUC) were similar in controls and T1D patients. In the total population, LPS-AUC correlated with triglyceride-AUC (r 0.244; p 0.034), cholesterol-AUC (r 0.245; p 0.033), and LDL-cholesterol-AUC (r 0.283; p 0.013). The strongest correlation was between LPS-AUC and insulin dose (r 0.528; p 0.001). ApoB-48, a specific chylomicron remnant marker, was elevated both at fasting [5.0 (3.7–8.8) vs. 3.6 (2.4–5.4 mmol/l); p 0.004] and AUC [109 (73–166) vs. 82 (49–111); p 0.008] in T1D patients compared to controls. In response to the first meal (8.00–12.00) iA\_ApoB-48 correlated positively with iA\_LPS in T1D patients (r 0.351; p 0.036). HDL cholesterol levels were similar in controls and T1D patients. NMR analysis of serum revealed that T1D patients had more large HDL particles than controls [AUC mean±SD: 4.7\*10<sup>-6</sup>±1.85\*10<sup>-6</sup> vs. 3.58\*10<sup>-6</sup>±1.82\*10<sup>-6</sup>; p 0.011]. This was supported by higher activity of factors involved in HDL-remodeling in T1D patients compared to controls: PLTP [AUC: 67910 (58431–75158) vs. 50903 (46274–75158); p<0.001] and CETP [AUC: 280 (233–313) vs. 248 (210–277); p 0.004]. Paraonase (PON1), an antioxidative enzyme bound to HDL, was lower in T1D patients than in controls [AUC: 191 (135–470) vs. 445 (167–655); p 0.021].

**Conclusion:** In this study we show that consecutive high fat meals do not lead to significant LPS accumulation. Chylomicron metabolism differed between T1D patients and controls, implying possible future vascular problems since ApoB-48 is a risk factor for cardiovascular disease. However, no obvious association between ApoB-48 and LPS was observed. Interestingly T1D patients had a higher proportion of large HDL particles and higher PLTP activity, indicating a potentially accelerated LPS clearance. PON1 activity on the contrary was decreased in T1D vs. controls, again suggesting differences in HDL subpopulations and their composition. To conclude metabolic en-

dotoxemia may be more central in patients with complications such as the metabolic syndrome or diabetic kidney disease.

Supported by: Stockmann fund, Liv och Hälsa, Diabetestutkimussäätiö, Novo Nordisk fund

## 328

### Does intrauterine exposure to maternal diabetes accelerate onset of type 1 diabetes in offspring? Results from the T1DGC

A.M. Wägner<sup>1,2</sup>, A. Santana<sup>3</sup>, J.C. Wiebe<sup>1</sup>, M. Hernández<sup>4</sup>, F.J. Nóvoa<sup>1,5</sup>, D. Mauricio<sup>4,6</sup>, T1DGC;

<sup>1</sup>Endocrinology, Complejo Hospitalario Universitario Insular Materno-Infantil, Las Palmas de Gran Canaria, <sup>2</sup>Departamento de Ciencias Médicas y Quirúrgicas, Universidad de Las Palmas de Gran Canaria (ULPGC),

<sup>3</sup>Departamento de Matemáticas y Estadística, ULPGC, Las Palmas de Gran Canaria, <sup>4</sup>Endocrinology, Hospital Arnau de Vilanova, Lleida, Spain,

<sup>5</sup>Ciencias Médicas y Quirúrgicas, Universidad de Las Palmas de Gran Canaria, <sup>6</sup>Institut de Recerca Biomedica, Lleida, Spain.

**Background and aims:** The T1DGC included families with at least 2 siblings with T1D. We previously showed that male gender, risk HLA and negative antibodies were associated with early onset of the disease. The aim of this study was to assess the role of maternal factors on the age of T1D onset in their offspring in the T1DGC dataset.

**Materials and methods:** Two approaches were made for data analysis: multivariate analysis for the search of predictors in the whole dataset and subgroup analysis of children whose mother had diabetes. Early onset of the disease was defined as onset in the lowest tertile (<6 years), whereas childhood-onset diabetes was defined as that diagnosed before the age of 15. Statistical analysis was performed using “R”. To assess the predictors of early and childhood onset, a multivariate regression analysis was performed, including gender, time since diagnosis, antibody positivity, presence of other autoimmune diseases and number of risk and protective HLA haplotypes, maternal age, and (first) birth order as independent variables. High-risk and protective haplotypes were defined according to a previous report from the T1DGC. Mothers with diabetes were identified and classified, both according to type of diabetes and to whether they were diagnosed before or after the birth of their first affected child. Age of onset of the first child with diabetes was compared in the different groups using Wilcoxon-Mann-Whitney’s test.

**Results:** Data including unequivocal HLA haplotypes was available from 2663 families. Median (range) age of onset of the disease was 9(0–49) years. In multivariate analysis, maternal age (p<1.25\*10<sup>-13</sup>) was a significant predictor of both early and childhood onset of the disease, whereas first birth order (p<0.013) was negatively associated. The previously described markers were also confirmed (data not shown). A total of 3228 mothers were identified. Their distribution into the different groups, maternal age at the time of birth of the first affected child and his/her age of onset are shown in the table.

	No diabetes	T1D-before	T1D-after	T2D-before	T2D-after
N	2995	53	31	21	128
Maternal age	26.3(4.7)	26.4(4.1)	23.1(3.5)	29.9(6.1)	26.1(4.7)
Age of onset of T1D	11.0(7.7)	7.8(5.8)*	12.4(8.8)	8.4(5.7)**	13.9(9.6)#

\*p<0.02 compared with no diabetes and T1D-after

\*\*p<0.02 compared with T2D-after

#p<0.002 compared with no diabetes

**Conclusion:** Both increasing maternal age and advanced birth order (after first) are associated with an earlier onset of T1D. Intrauterine exposure to hyperglycaemia also seems to be associated with earlier onset of the disease in offspring, although further analyses are needed to adjust for its interaction with maternal age.

Supported by: NIDDK (DK-62418) and JDRF, ISCIII, EFSD/JDRF/Novo Nordisk grant

## 329

**Diabetes and influenza-attributable health care utilisation: a population-based cohort study**D. Lau<sup>1</sup>, D.T. Eurich<sup>1</sup>, S.R. Majumdar<sup>2</sup>, A. Katz<sup>3</sup>, J.A. Johnson<sup>1</sup>;<sup>1</sup>Department of Public Health Sciences, University of Alberta, Edmonton,<sup>2</sup>Department of Medicine, University of Alberta, Edmonton, <sup>3</sup>Department of Family Medicine, University of Manitoba, Winnipeg, Canada.

**Background and aims:** Guidelines recommend routine vaccination against seasonal influenza in all patients with diabetes. Since vaccinations are already recommended in the elderly, these guidelines single out non-elderly adults with diabetes. However, there is limited evidence that this group actually suffers from increased influenza-related morbidity and mortality compared with otherwise healthy, non-elderly adults. We therefore compared population-based rates of influenza-attributable illness in adults with and without diabetes.

**Materials and methods:** We performed a cohort study using administrative claims data from Manitoba, Canada, between 2000 to 2008. These data capture all drugs and services provided by universal health care. All adults (18 years and older) with diabetes were identified and matched with up to two non-diabetic controls. Our outcomes were physician visits and hospitalizations for influenza-like illness (ILI); and hospitalizations for pneumonia and influenza (PI), and all causes (ALL). Using multivariate Poisson regression to adjust for prior health status, comorbidities, vaccinations, and seasonal trends, we estimated differences in the influenza-attributable rates of each outcome for patients with and without diabetes during periods of known circulating influenza. We performed analyses for all adults, and then separately for the non-elderly (< 65 years) and elderly (>= 65 years).

**Results:** We included 1.17 million person-years of follow-up among 239012 subjects. Of 82027 subjects with diabetes, 51154 (62%) were non-elderly. During the study period, there were 412043 physician visits or hospitalizations for ILI, 7338 PI hospitalizations, and 134799 all-cause hospitalizations. In those with diabetes, circulating influenza increased event rates by (%-relative increase [95% CI]): 14% [12–15%] (ILI), 20% [11–30%] (PI), and 6% [4–8%] (ALL). For those without diabetes, influenza increased event rates by 13% [12–14%] (ILI), 8% [–1–19%] (PI), and 7% [4–9%] (ALL). Formal tests of interaction confirmed a difference in the effect of influenza between those with and without diabetes only for PI hospitalizations (IRR = 1.11 [1.00, 1.22],  $p=0.04$ ). In our age-stratified analyses, we only detected increased rates of influenza-attributable ILI among the elderly (IRR = 1.03 [1.01, 1.05],  $p=0.01$ ) and all-cause hospitalization among the non-elderly (IRR = 1.06 [1.00, 1.11],  $p=0.04$ ). Thus, non-elderly adults with diabetes were significantly more likely than similar adults without diabetes to have influenza-attributable all-cause hospitalizations. This translated into an 54, potentially preventable hospitalizations (approximately 1 per 1000 adults) each year.

**Conclusion:** In non-elderly adults with diabetes, influenza is associated with a relative increase in hospital admissions, for any cause, during influenza season. Nevertheless, the absolute increase in risk is small, and the rationale for targeting vaccinations for non-elderly adults with diabetes depends primarily on the balance of costs and effectiveness of vaccine.

Supported by: CIHR (MOP-119316)

## 330

**Enterovirus infection in Tunisian patients with type 1 diabetes mellitus**I. Slim<sup>1</sup>, I. Bousaid<sup>2</sup>, R. Zemni<sup>2</sup>, L. Gueddah<sup>2</sup>, K. Ach<sup>1</sup>, M. Kacem<sup>1</sup>, A. Maaroufi<sup>1</sup>, M. Chaieb<sup>1</sup>, L. Chaieb<sup>1</sup>, F. Ben Hadj Slama<sup>2</sup>;<sup>1</sup>Department of Endocrinology and Diabetology, Farhat Hached University Hospital, <sup>2</sup>Department of Immunogenetics, Ibn Jazzar Faculty of Medicine, Soussa, Tunisia.

**Background and aims:** Type 1 diabetes mellitus (T1DM) is a metabolic disease with serious degenerative complications. The disease is due to progressive autoimmune beta pancreatic islet cells destruction. Environmental factors, especially viruses, are believed to contribute to the pathogenesis of T1DM. Enteroviral (EV) infections have long been suspected in having a role in  $\beta$  cell damaging process and therefore leading to the onset of clinical T1DM. This study was designed to assess a possible link between EV infection and T1DM.

**Materials and methods:** Plasma samples were collected from 95 patients with T1DM (36 children and 59 adults) at age ranging from 5 to 40 years. They were classified according to the course of the disease into two groups: the children (5 children newly diagnosed with T1DM and 31 children with an

older disease) and the adult group which included 16 patients who were at the onset of the disease and 43 patients previously diagnosed with T1DM. Viral RNA was assessed using a highly sensitive nested RT-PCR of the 5' untranslated region (5'UTR) of the genome of EV. Enteroviral infection investigation included as well as control group ( $n = 141$ ) who were clinically free of diabetes or any autoimmune diseases.

**Results:** EV-RNA was detected more frequently in plasma from diabetic patients than in plasma of controls (31,6% vs. 7,8%,  $p < 0,0001$ ; OR = 5,45, IC95% 2,44–12,43). The EV infection was found with a higher frequency in children than in adults. The frequency of positive signals corresponding to enteroviral sequence amplifications were higher in newly diagnosed T1DM children (3/5, 60%) than in their corresponding control children (7/55, 12,7%,  $p = 0,028$ ; OR = 10,29, IC95% 1,10–112,33). We also showed that the frequencies of viral RNA reached the limit of significance in adults recently diagnosed ( $p = 0,075$ ; OR = 4,73, IC95% 0,73–29,60). The statistical analysis of the nested RT-PCR data demonstrates that there was no significant difference in the prevalence of EV infection between newly diagnosed T1DM and previously diagnosed T1DM patients in children and adult groups (3/5, 60%; 18/31, 58,1%,  $p = 1,00$ ; 3/16, 18,8%, 6/43, 14%,  $p = 0.69$ , respectively) although there was a tendency to a decrease of the frequency of EV infection in T1DM older than ten years. GAD and IA-2 antibodies were not significantly associated to EV infection ( $p = 1,00$ ).

**Conclusion:** The present study demonstrates that EV RNA sequences are detected in the plasma from patients at the onset or in the course of T1DM more often than in non diabetic subjects and suggests that EV infection could be involved in the pathogenesis of the T1DM.

## 331

**Autophagy in Coxsackievirus infected pancreatic beta cells**E. Töpfer<sup>1</sup>, K.-P. Knoch<sup>1</sup>, A. Petzold<sup>1</sup>, C. Wegbrod<sup>1</sup>, A. Sönmez<sup>1</sup>, M. Roivainen<sup>2</sup>, M. Solimena<sup>1</sup>;<sup>1</sup>Molecular Diabetology, University of Technology Dresden, Germany,<sup>2</sup>National Institute for Health and Welfare, Helsinki, Finland.

**Background and aims:** Both genetic disposition and environmental factors contribute to the pathogenesis of Type 1 diabetes (T1D). Human Enteroviruses (HEV), including Coxsackieviruses B (CVB), in particular, have been linked to an increased incidence of T1D. Several studies have shown a connection between enterovirus infection and autophagy. The latter process is key for maintenance of cell homeostasis through the degradation and recycling of intracellular organelles and for surveillance of viral infection. Some viruses, however, can arrest autophagosome development and even manipulate autophagy to enable their own lifecycle. Hence, we investigated the involvement of autophagy in the replication of CVB5 in mouse insulinoma MIN6 cells.

**Materials and methods:** MIN6 cells stably transfected with the autophagy marker GFP-LC3 were infected with a mouse cell adapted (MCA) CVB5 DS-1. Four days after infection induction of autophagy and virus replication were analyzed by western blotting for GFP-LC3 and the capsid viral protein (VP1), while GFP-LC3+ compartments (LC3-AC) were isolated from both infected or starved cells and further evaluated for their content.

**Results:** CVB5 infected MIN6 cells expressed higher levels of GFP-LC3B-II and VP1 and both proteins were detected in isolated LC3-AC. LC3-AC isolated from CVB5 infected MIN6 cells contained lower amounts of the ER-marker calnexin compared to not infected cells cultured either in normal conditions or starved. Conversely, LC3-AC from CVB5 infected cells contained higher levels of the membrane granule marker ICA512-TMF, whereas its precursor pro-ICA512 was more abundant in LC3-AC isolated from not infected control or starved cells.

**Conclusion:** The positive correlation between GFP-LC3B-II and VP1 expression suggests that autophagy is implicated in the replication of CVB5. This hypothesis is corroborated by the enrichment of VP1 in LC3-AC. Enrichment of pro-ICA512 in LC3-AC from control or starved cells, and of ICA512-TMF in LC3-AC from CVB5 infected cells further suggest that autophagosomes of control or starved cells use mainly the ER as a membrane source, whereas those of CVB5 infected cells utilize secretory granule membranes.

## PS 008 Monogenic forms of diabetes

### 332

#### Integration of biomarkers and clinical characteristics provides the best method for identifying patients with MODY

B.M. Shields<sup>1</sup>, T.J. McDonald<sup>2</sup>, K.R. Owen<sup>3</sup>, M.T. Malecki<sup>4</sup>, R.E.J. Besser<sup>1</sup>, A.G. Jones<sup>1</sup>, S. Ellard<sup>2</sup>, A.T. Hattersley<sup>1</sup>;

<sup>1</sup>Peninsula Medical School, University of Exeter, UK, <sup>2</sup>Royal Devon and Exeter NHS Foundation Trust, Exeter, UK, <sup>3</sup>Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, UK, <sup>4</sup>Department of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland.

**Background and aims:** Maturity-onset diabetes of the young (MODY) is a rare, young-onset form of diabetes, often misdiagnosed as Type 1 diabetes (T1D) or Type 2 diabetes (T2D), resulting in inappropriate management. A number of biomarkers have been proposed to aid identification of patients with MODY and clinical characteristics (age at diagnosis, BMI, HbA<sub>1c</sub>, parent with diabetes, treatment) are useful when combined in a clinical prediction model. We aimed to determine if combinations of biomarkers and clinical criteria improves the diagnostic accuracy for detecting MODY in patients diagnosed under 45.

**Materials and methods:** We measured plasma C-peptide and GAD and IA-2 antibodies on patients insulin treated within 6 months of diagnosis (144 T1D, 71 MODY), and hsCRP and HDL cholesterol in patients not insulin treated within 6 months of diagnosis (118 T2D, 216 MODY). Probability of MODY for each patient was derived using the clinical prediction model. Discriminative ability of the biomarkers/criteria was assessed using Receiver Operating Characteristic (ROC) curves.

**Results:** In patients insulin treated within 6 months of diagnosis, C-peptide > 80 pmol/L and absence of GAD/IA-2 antibodies had 90% sensitivity, 93% specificity for MODY. The addition of clinical characteristics provided a minor improvement in diagnostic accuracy (ROC AUC 0.95 v 0.99,  $p < 0.001$ ; perfect test AUC = 1). In patients not insulin treated within 6 months of diagnosis, hsCRP < 0.75 mg/L and HDL > 1.12 mmol/L had 56% sensitivity and 87% specificity. The addition of clinical characteristics greatly improved diagnostic accuracy (ROC AUC 0.81 v 0.98,  $p < 0.0001$ ).

**Conclusion:** The use of multiple biomarkers outperforms single biomarkers for predicting MODY. Excellent discrimination between MODY and Type 1/Type 2 diabetes is achieved when biomarkers are used in combination with clinical characteristics.

Supported by: EU FP7 HEALTH-F2-2008-223211

### 333

#### Assessment of new clinical criteria to search for HNF1A MODY in patients with initial diagnosis of type 1 or type 2 diabetes mellitus

M. Szopa, M. Grzanka, B. Kiec-Wilk, N. Nowak, B. Matejko, M.T. Malecki, T. Klupa;  
Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland.

**Background and aims:** The most common form of maturity-onset diabetes of the young (MODY) is caused by mutations in the hepatocyte nuclear factor 1A (HNF1A) gene. It has been established that patients with HNF1A MODY are very sensitive to sulphonylureas (SU) and can also discontinue insulin treatment. However, most of patients with this monogenic diabetes are misdiagnosed as having type 1 (T1DM) or type 2 (T2DM) diabetes mellitus and hence receive non-optimal treatment.

**Materials and methods:** The aim of our study was to test new clinical criteria for selecting patients for HNF1A MODY molecular testing: a) for patients initially diagnosed as T2DM, the selection criteria were as follows - effective SU based oral treatment for more than 15 years and body mass index < 30 kg/m<sup>2</sup>; b) for patients with T1DM clinical diagnosis, we selected individuals currently treated with continuous subcutaneous insulin infusion (CSII) - for those individuals it is possible to obtain very precise and dependable information concerning insulin dosing based on insulin pump downloads. Selection criteria included total daily insulin requirement (TDIR) of less 0.3 IU/Kg at least five years after diabetes diagnosis and basal insulin requirement of less than 30% of TDIR.

**Results:** We analyzed medical records of 664 diabetic patients, 524 clinically diagnosed as T2DM and 159 classified as T1DM. Based on examined clinical

criteria, we selected 14 subjects from patients with T2DM clinical diagnosis; eventually 11 of them were available for the HNF1A gene sequencing. In one patient from this group, we have found a large deletion including nucleotides 1380-1408, which is a causative variant of diabetes in this subject (9% of sequenced cases). Among patients with T1DM clinical diagnosis who were treated with CSII and who met selection criteria, the previously described diabetes-related mutation in exon 4 (P291fsinsC) was found in one individual (50% of analyzed cases).

**Conclusion:** In conclusion, we present new clinical criteria which may be helpful in pre-selecting patients for molecular testing in search for the HNF1A MODY.

Supported by: CEED3 FP7 EU Grant

### 334

#### The incretin effect in patients with maturity onset diabetes of the young

S.H. Østoft<sup>1,2</sup>, J.I. Bagger<sup>1,2</sup>, T. Hansen<sup>3</sup>, O. Pedersen<sup>3</sup>, J.J. Holst<sup>2,3</sup>, F. Knop<sup>1</sup>, T. Vilsbøll<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, Diabetes Research Division, Hellerup,

<sup>2</sup>Department of Biomedical Sciences, Panum Institute, Copenhagen, <sup>3</sup>Novo Nordisk foundation Center for Basic Metabolic Research, Copenhagen, Denmark.

**Background and aims:** Maturity onset diabetes of the young (MODY) is a clinically and genetically heterogeneous subgroup of non-autoimmune diabetes, constituting about 1-2% of all diabetes. The incretin effect (the enhancement of glucose-induced insulin secretion following oral glucose tolerance test (OGTT) compared to isoglycaemic intravenous glucose infusion (IIGI)) is unclear in MODY patients. In the present study we studied the incretin effect in patients with MODY2 (glucokinase gene mutations) and MODY3 (hepatocyte nuclear factor 1a gene mutations) and in a group of matched control subjects.

**Material and methods:** Ten MODY3 patients (age: 31±3 years (mean±SEM); body mass index (BMI): 24±1 kg/m<sup>2</sup>; fasting plasma glucose (FPG): 8.4±0.8 mmol/L; HbA<sub>1c</sub>: 7.0±0.3%), 9 MODY2 patients (age: 43±5 years; BMI: 24±2 kg/m<sup>2</sup>; FPG: 7.3±0.3 mmol/L; HbA<sub>1c</sub>: 6.7±0.2%) and 9 control subjects (age: 41±5 years; BMI: 24±1 kg/m<sup>2</sup>; FPG: 5.1±0.2 mmol/L; HbA<sub>1c</sub>: 5.3±0.1%) were examined on 2 separate occasions: 4h 50 g-OGTT and IIGI.

**Results:** MODY3 patients exhibited more severe glucose intolerance (evaluated from area under curve during OGTT) than MODY2 patients (3,041±302 mmol/L×4h vs. 2,139±59 mmol/L×4h,  $p=0.0126$ ) compared to the control subjects (1,351±20 mmol/L×4h;  $p<0.001$  and  $p<0.001$ , respectively). Isoglycaemia was obtained using 37±4 g and 30±3 g of glucose during IIGIs in patients with MODY3 and MODY2, respectively ( $p=NS$ ), and 24±2 g in control subjects ( $p=0.01$  and  $p=0.11$ , respectively). The incretin effect [ $100\% \times (AUC_{C-peptide, OGTT} - AUC_{C-peptide, IIGI}) / AUC_{C-peptide, OGTT}$ ] amounted to 17±5% and 26±3% in patients with MODY3 and MODY2, respectively ( $p=NS$ ) and 36±4% in the control subjects ( $p=0.018$  and  $p=NS$ , respectively).

**Conclusion:** Our data suggest that MODY2 patients are the first group of diabetes patients ever seen with normal incretin effect. In contrast, MODY3 patients were characterized by an impaired incretin effect when compared to control subjects. The differences are most likely explained by differences in the etiology and glucose tolerance between MODY3 and MODY2 patients.

Clinical Trial Registration Number: NCT01342939

Supported by: The Novo Nordisk Foundation Center

### 335

#### Whole exome sequencing identifies CUL9 as a MODY-X gene

N. Iwasaki<sup>1,2</sup>, A. Saito<sup>3</sup>, T. Furukawa<sup>2</sup>, M. Takizawa<sup>1</sup>, R. Fujimaki<sup>1</sup>, M. Ogata<sup>1</sup>, Y. Uchigata<sup>1</sup>;

<sup>1</sup>Diabetes Center, Tokyo Women's Medical University, <sup>2</sup>Institution of Integrated Medical Science, Tokyo Women's Medical University, <sup>3</sup>STAGEN, Tokyo, Japan.

**Background and aims:** Whole exome sequencing is known to be a powerful tool for identifying the causative mutations in Mendelian diseases. We have previously determined the regions of Max NPL > 3.0 on chromosomes 6, 7 and 22 by linkage analysis, in one Japanese MODY family that showed > 60% power to detect NPL score > 2.0. However, regions detected were still too broad to screen. The aim of this study is to identify the causative variants of MODY-X by combining the information with whole exome sequencing on the linkage data.



**Materials and methods:** The MODY-X family with three generations consisted of 31 members was studied. Genomic DNA was available from 31 subjects. Affection status was confirmed by 75g OGTT, if the individual was not screened for diabetes mellitus. We performed whole exome sequencing for four individuals including the proband, the daughter and the nephew with MODY and the spouse of the proband who was not affected. Exomes (38 Mb of genomic sequence) of four individuals using the SureSelect human exome kit (Agilent) were subsequently sequenced using a SOLiD sequencing slide (Life Technologies). Bioscope was used for detecting SNPs and insertion-deletions. Polyphen and SIFT were used for selecting the variants. The causative genes were defined as to satisfy all four criteria; 1) located in the protein coding region, 2) heterozygous only in patients, 3) unknown SNP (not deposited in dbSNP nor in 1000 Genome), and 4) matched with linkage results.

**Results:** On average, 50% of exome in the proband and 85% of exome in the remaining individuals were covered more than tenfold, and 156,959 genetic variants were identified including 20,162 non-synonymous changes. The number of the variants fulfilled the criteria of 1), 2), 3) and also located on chromosomes 6, 7 and 22 were 41. The screening by Polyphen score > 0.6 and/or SIFT score 2.0 selected CUL9 and HMGCL1 those located on the peak on chromosome 6. However, HMGCL1 was rejected by Sanger sequence, and only CUL9 satisfied both Polyphen and SIFT scores.

**Conclusion:** We identified CUL9 as the MODY-X gene based on the linkage results in combination with a massively parallel sequencing of whole exome, though the functional properties needs to be elucidated.

*Supported by: The grant from the Ministry of Science, Culture and Sport of Japan*

### 336

#### Identification of a new mutation of C1SD2 gene in siblings with Wolfram syndrome 2

M. Rondinelli<sup>1</sup>, F. Novara<sup>2</sup>, V. Calcaterra<sup>2</sup>, L. Bucciarelli<sup>1</sup>, V. Valdes<sup>1</sup>, D. Larizza<sup>2</sup>, O. Zuffardi<sup>2</sup>, S. Genovese<sup>1</sup>

<sup>1</sup>IRCCS Multimedica, Sesto San Giovanni, Italy, <sup>2</sup>IRCCS San Matteo University of Pavia, Italy.

**Background and aims:** Wolfram syndrome (WFS; MIM 222300) is an autosomal recessive neurodegenerative disorder. Affected individuals suffer by juvenile-onset insulin-requiring diabetes mellitus. Other neurological and endocrine manifestations include optic atrophy, sensorineural deafness, psychiatric illnesses, diabetes insipidus, renal-tract abnormalities and bladder atony. One gene for the disorder, WFS1, on chromosome 4p16.3, encodes a transmembrane protein, Wolframin, located in the endoplasmic reticulum (ER), which plays a role in calcium homeostasis. A second gene, WFS2 (C1SD2), on chromosome 4q22-24 encodes for ERIS (endoplasmic reticulum intermembrane small protein). An extensive phenotypic analysis showed additional symptoms in individuals with WFS2, such as significant bleeding tendency, as well as a defective platelet aggregation with collagen; a considerable number of patients with WFS2 had also peptic ulcer disease. We performed genetic analysis of an Italian family composed by two sisters affected by WFS and their unaffected parents. The probands suffer by the following abnormalities, reported in chronological order according to diagnosis: peptic ulcer, diabetes mellitus, diabetes insipidus, sensorineural deafness, amenorrhoea, optic atrophy and bladder atony.

**Materials and methods:** EDTA blood was obtained and genomic DNA was isolated by using QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's protocol. The three C1SD2 coding exons were amplified by PCR using Go Taq(R) Flexi DNA Polymerase (Promega). Sequence primers and experimental conditions are available on request.

**Results:** C1SD2 (NM\_001008388) sequencing analysis revealed a novel homozygous substitution (cDNA: NM\_001008388.4:c.103+1G>A, DNA Level: Chr4(GRCh37):g.103790345G>A) in the donor splice site of intron 1 in the probands. Both parents resulted heterozygous for the same mutation.

**Conclusion:** C1SD2 is the second member of the gene family containing the CDGSH iron sulfur domain. There are currently three members in this gene family: C1SD1 (synonyms ZCD1, mitoNEET), C1SD2 (synonyms ZCD2, Noxp70, and Miner1) and C1SD3 (synonym Miner2). C1SD2 gene has been identified as the second causative gene associated with WFS. Moreover, recent studies demonstrated that C1SD2 deficiency causes mitochondrial-mediated phenotypic defects in mice and C1SD2 knockout mice exhibit many clinical manifestations of WFS patients including early-onset degeneration of central (e.g., optic) and peripheral (e.g., sciatic) nerves and premature death, as well as impaired glucose tolerance. The homozygous substitution identified in C1SD2 gene of our patients maps in the donor splice site of in-

tron 1 (c.103+1G>A). This mutation was not found in the single nucleotide polymorphism database (dbSNP; www.ncbi.nih.gov/projects/SNP/). Further study of parents revealed that they carried the mutation in a heterozygous state. Comparative genomics analysis, by aligning nucleotide sequences of different species, such as chimp, rhesus, cat and mouse showed that the site of identified mutation is conserved during evolution. Functional predictions of the homozygous mutation were performed using bioinformatics methods such as Alamut and Mutation Taster (<http://www.mutationtaster.org/>).

### 337

#### Localisation of hepatocyte nuclear factor-4alpha in the nucleolus and nucleus is regulated by its C-terminus

M. Ogata, N. Iwasaki, R. Fujimaki, M. Takizawa, Y. Uchigata;

Tokyo Women's Medical University, Diabetes Center, Tokyo, Japan.

**Background and aims:** Mutations in hepatocyte nuclear factor-4α (HNF4α) lead to various diseases, among which C-terminal deletions of HNF4α are exclusively responsible for maturity onset diabetes of the young 1 (MODY1). MODY is an autosomal dominant disease characterized by a primary defect in insulin response to glucose, suggesting that the C-terminus of HNF4α is important for pancreatic β-cell function. To clarify the role of the C-terminus of HNF4α, changes in cellular localization and the binding ability to its regulator were examined, specifically in the region containing Q268, which deletion causes MODY1.

**Materials and methods:** Cellular localization of mutant HNF4α were examined in monkey kidney 7 (COS7), Chinese hamster ovary(CHO), rat insulinoma (INS-1) and mouse insulinoma (MIN6) cells, and their binding activity to other proteins were examined by fluorescence resonance energy transfer (FRET) in COS7 cells.

**Results:** Although wild-type HNF4α was localized in the nucleoplasm in transfected cultured cells, Q268X-HNF4α was located predominantly in the nucleolus. Deletion analysis of the C-terminus of HNF4α showed that the S337X-HNF4α mutant, and other mutants with shorter amino acid sequences (S337-K194), were mostly localized in the nucleolus. HNF4α mutants with amino acid sequences shorter than the W192X-HNF4α mutant gradually spread to the nucleoplasm in accordance with their lengths. The A250X-HNF4α mutant was capable of causing the accumulation of HNF4α or the small heterodimer partner (SHP), one of the HNF4α regulators, in the nucleolus. However, the R154X-HNF4α mutant did not have binding ability to wild-type HNF4α or SHP, and thus was seen in the nucleus.

**Conclusion:** The C-terminus sites might play a key role in facilitating the nucleolar and subnucleolar localization of HNF4α.

*Supported by: MEXT*

### 338

#### Inefficient preproinsulin translocation links to late onset diabetes

M. Liu<sup>1</sup>, H. Guo<sup>1</sup>, Y. Xiong<sup>1</sup>, R. Lara-Lemus<sup>1</sup>, S.-O. Shan<sup>2</sup>, P. Arvan<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, University of Michigan, Ann Arbor,

<sup>2</sup>Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, USA.

**Background and aims:** In pancreatic beta cells, insulin biosynthesis begins as a precursor, preproinsulin (PPI), within the cytosol. To enter the secretory pathway, the signal peptide (SP) of newly synthesized PPI recognized by signal peptide recognition particle (SRP) drives PPI to and across the endoplasmic reticulum (ER) membrane. Recently, two SP mutations, R6C and R6H, close to the amino terminus of PPI were reported to cause late onset diabetes in humans. The mutations decrease SP positive charge, which may affect ER targeting/translocation efficiency of PPI. However, a new study has suggested that the protein encoded by these mutations could still be targeted to insulin secretory granules, raising the question of how these SP mutations could cause diabetes.

**Materials and methods:** We use metabolic labeling to examine co-translational translocation and SP cleavage of wild-type and mutant PPIs.

**Results:** Upon metabolic labeling of newly synthesized PPIs, we found that, although the mutant SPs could be recognized by SRP and targeted to the ER membrane, up to 50% of mutant PPIs failed to fully translocate into the ER lumen, which resulted in a ~50% decrease of insulin production from the mutant PPIs in transfected pancreatic beta cells. Interestingly, this translocation defect was fully rescued by restoring positive charge to the mutant SPs, indicating that positive charge within the SP plays an important role in

efficiency of PPI translocation. All patients carrying these mutations are heterozygous, yet, the insulin haploinsufficiency cannot itself account for diabetes. To examine the molecular mechanisms of beta cell failure associated with these mutants, we co-expressed mutant and wild-type (WT) PPIs. We found that, unlike other mutant (pre)proinsulins associated with the syndrome of Mutant INS-gene-induced Diabetes of Youth (MIDY), these two mutant PPIs did not affect normal trafficking and insulin production of co-expressed WT proinsulin. Instead, the translocation defect led to accumulation of the untranslocated PPIs in the cytosol, which might be toxic to pancreatic beta cells, leading to late onset diabetes.

**Conclusion:** Our study suggests that preproinsulin SP mutations associated with late onset diabetes are mistargeted and accumulated in the cytosol. Manipulating cellular events that accelerate degradation and prevent aggregation of untranslocated PPIs may protect beta cells from the toxicity of these mutants.

*Supported by: NIDDK DK088856 to M.L.*

## PS 009 Diabetes, cardiovascular disease and mortality

339

### Coronary artery disease as a risk for developing type 2 diabetes mellitus

C.H. Saelly<sup>1,2</sup>, A. Vonbank<sup>3,1</sup>, P. Rein<sup>3,1</sup>, S. Beer<sup>3,1</sup>, H. Drexel<sup>1,4</sup>,

<sup>1</sup>VIVIT Institute, Feldkirch, Austria, <sup>2</sup>Private University of the Principality of Liechtenstein, Triesen, Liechtenstein, <sup>3</sup>Academic Teaching Hospital, Feldkirch, Austria, <sup>4</sup>Drexel University College of Medicine, Philadelphia, USA.

**Background and aims:** Diabetes mellitus is a major risk factor for coronary artery disease (CAD); whether conversely CAD confers an increased risk for diabetes has not been studied so far and is addressed in the present study.

**Materials and methods:** We prospectively recorded incident diabetes over 7.5 years in 506 consecutive non-diabetic Caucasian patients undergoing coronary angiography for the evaluation of stable CAD, covering 3795 patient years.

**Results:** During follow-up, diabetes was newly diagnosed in 107 patients, i.e. in 21.1% of the study population or in 2.8% per year. Patients with significant CAD (n=293) when compared to subjects who did not have significant CAD at the baseline angiography were at a 33% (p = 0.027) increased diabetes risk. However, the relationship between CAD and incident diabetes was attenuated and no longer statistically significant after adjustment for potential confounders including metabolic syndrome (MetS) status. The MetS as diagnosed according to the current consensus definition in turn was strongly predictive of diabetes, in particular when the more selective NCEP-ATP-III waist cut-off values were applied for its diagnosis (OR = 2.91 [1.83–4.64]; p < 0.001).

**Conclusion:** We conclude that albeit apparently not causally related to diabetes incidence, the presence of CAD indicates a strongly increased risk for incident diabetes. Repeated diabetes screening of coronary patients and targeted programs to prevent diabetes in these high-risk patients are warranted.

*Supported by: Jubiläumsfonds of the Austrian National Bank*

340

### Glycaemic status, diabetes, and cardiovascular risk: a new risk score for the aged Southern European population

R. Gabriel<sup>1</sup>, C. Brotons<sup>2</sup>, J. Muñoz<sup>3</sup>, J. Tuomilehto<sup>1</sup>,

<sup>1</sup>Clinical Epidemiology, Hospital Universitario La Paz, Madrid, <sup>2</sup>EAP Sardenya, Barcelona, <sup>3</sup>Universidad de A Coruña, A Coruña, Spain.

**Background:** The impact of diabetes mellitus (DM) on cardiovascular disease (CVD) risk is an important aspect to be considered in risk assessment. The European HEARTSCORE did not consider the impact of glycaemic status, anti-diabetic treatment and the age groups older than 70 years on the total CVD risk.

**Objective:** To develop a new CVD risk prediction tool, including information on glycaemic status, treatment of diabetes, to accurately estimate the individual cardiovascular risk in Southern Europe, where elderly people account for more than 25% of the total population, and have higher prevalence of DM than middle-aged groups.

**Methods and material:** The project assembled a pool of 7 Spanish cohort studies including middle-aged (30–70 years) and elderly individuals (>70 years). There were 11,800 persons free of CVD at baseline (5,413 men and 6,387 women) representing 108,569 person years of follow-up. DM was defined as FPG ≥ 7.0 mmol/L, random capillary FG ≥ 11.1 mmol/L or treatment with any anti-diabetic drug at baseline. Cox regression analyses were conducted to examine the contributions of the different variables to CVD forming the potential basis for the development of the CVD risk-score (ERICE-score).

**Results:** Overall prevalence of DM at baseline was 8.6% (8.8 in males, and 8.4 in females). A total of 1,214 cardiovascular events were identified, of which 633 were fatal. Age was the strongest risk factor for CVD. With regard to modifiable risk factors, in men, high SBP was the strongest predictive factor of CVD followed by DM and smoking with similar impact. In women, DM plays a crucial role followed by smoking and high SBP. Multivariate adjusted hazard ratios for CVD in people with treated DM, compared to non-diabetics, were 1.37 (95%CI:1.22–1.46) in men and 1.59 (1.30–1.69) in women. The contribution of high total-cholesterol levels to the CVD risk was only significant, both in men and women, in younger than 70 years.

**Conclusion:** Separate risk chart are given for treated diabetics, non-treated diabetics and non-diabetics, both for males and females, because the absolute risk increased with its combination, with the highest risk in men treated diabetics. The individual contribution of DM to the global CVD risk was also higher than total-cholesterol in this aged Southern European population.

*Supported by:* Fondo de Investigación Sanitaria. Instituto de Salud Carlos III. Spain

## 341

### Excess mortality in the English National Diabetes Audit is greater in younger people as compared with older people who have both type 1 and type 2 diabetes

B. Young<sup>1</sup>, D. Eayres<sup>2</sup>, A. Uddin<sup>2</sup>, J. Henderson<sup>2</sup>,

<sup>1</sup>Salford Royal Hospital, Salford, <sup>2</sup>HSCIC, Leeds, UK.

**Background and aims:** Data from English National Diabetes Audit (NDA) was investigated to determine whether age, sex and type of diabetes are related to excess mortality in diabetics.

**Materials and methods:** Using unique person identifiers (NHS numbers) from the 1.7 million people with diabetes included in the 2007/8 NDA the UK Office of National Statistics identified 49,282 deaths during the year beginning 1 November 2008. The diabetes cohort's mortality was investigated using age and sex-specific mortality rates, directly age-standardised rates (DSR) and indirectly age-standardised mortality ratios (SMR), with England 2009 as the standard population.

**Results:** The crude death rate for all people with diabetes was 3,553 per 100,000 per year (background England rate 886). People with type 1 diabetes had the highest mortality with a DSR of 2,016 compared to 1,462 for people with type 2 diabetes and 886 for the background England population. The SMRs suggest that, compared with the background mortality rates, there are approximately 2.5 times as many deaths in people with type 1 diabetes and 1.5 times as many in people with type 2 diabetes. In total there were >16 thousand more deaths of people with diabetes than would have occurred if their mortality risk was equal to that of the general population. Extrapolating this to include people with diabetes not in the audit, suggests that in 2008–9 about 21 thousand excess deaths occurred in people with diabetes in England. Compared to the background population both males and females with type 1 diabetes were 2.5 times more likely to die while for type 2 diabetes the corresponding rates were 1.4 for males and 1.5 for females. In all instances this excess mortality was inversely related to age: in the 15–34 age group, females with type 1 diabetes had mortality approximately 9 times the background female rate while in males it was 4 times (largely because the background mortality in young males is much higher than for females); for type 2 diabetes the figures are approximately 6 and 3.6 times respectively; in the 85+ age group all of the ratios drop to less than 2. Compared to the general population there was less excess male mortality in type 1 diabetes: no excess in the 15–34 age group and a gradual increase to 1.3 times in the over 85s; by contrast for type 2 diabetes excess male mortality is constant over all age groups at approximately 1.2 times the female rate.

**Conclusion:** Compared with the general population the contemporary risk of death in people with diabetes is higher at all ages in both sexes and in both Type 1 and Type 2 diabetes but the relative risk is greatest in the young and in females with Type 1 diabetes.

*Supported by:* HQIP

## 342

### Other causes of death - are there any unexpected specific mortality rates in diabetes patients?

S. Ioacara<sup>1</sup>, C. Guja<sup>1</sup>, C. Ionescu-Tirgoviste<sup>1</sup>, S. Fica<sup>2</sup>, S. Martin<sup>2</sup>, S. Sabau<sup>3</sup>, C. Tiut<sup>4</sup>;

<sup>1</sup>"I. Pavel" Outpatient Clinic, Bucharest, Romania, <sup>2</sup>"Elias" Hospital, Bucharest, Romania, <sup>3</sup>"Tokai" University, Sapporo, Japan, <sup>4</sup>"University" Hospital, Bucharest, Romania.

**Background and aims:** Almost all research papers on mortality are focused on all-cause, cardiovascular, cancer, diabetes-related or chronic kidney disease. The remaining causes of death are designated as "other causes" and usually overlooked. This study investigated the mortality from other causes of death in type 2 diabetes (T2DM).

**Materials and methods:** All diabetes subjects residing in a major urban area and its surroundings were included at the moment of their first diabetes pre-

scription between 01/01/2001 and 12/31/2008 (n=82763). Subjects younger than 40 years (n=4940), insulin treated at baseline (n=11936), follow-up <6 months (n=760), and missing essential data (n=2547) were excluded. All 62580 remaining T2DM subjects oral-treated at baseline were followed-up for all-cause / other causes mortality until 12/31/2010, by cross-linking with the National Institute of Statistics mortality database, based on International Classification of Diseases 10th revision (ICD10). Deaths from "other causes" (ICD10 codes: A00-B99, D10-E09, E15-H95, J00-N16, N20-T99) were divided into 5 categories according to medical relevance, and 11 subcategories based on the availability of at least 10 deaths in both men and women. Control group was constructed using the general population data adapted to the yearly age and sex distribution of the diabetes group. Standardised mortality ratios (SMR) were calculated.

**Results:** The mean age at baseline was 62±10.2 years (61±10.3 years in men and 62.9±10 years in women, p<0.001), with a mean follow-up of 6.4±2.8 years (6.3±2.8 years in men and 6.5±2.8 years in women, p<0.001) leading to 402,243.1 person-years of follow-up and 13459 all-cause deaths (1705 deaths from other causes). The control group contributed 98,202,901 person-years and 3,304,629 all-cause deaths (512742 deaths from other causes). SMR for the five major categories of death from "other causes" are presented in table 1.

Table 1

Cause of death (ICD10)	Males SMR (CI95, p)	Females SMR (CI95, p)
Infectious (A00-B99)	0.29 (0.182-0.44, p<0.001)	0.816 (0.499-1.265, p=0.387)
Respiratory (J00-J99)	0.568 (0.5-0.643, p<0.001)	1.087 (0.957-1.229, p=0.192)
Digestive (K00-K93)	1.015 (0.925-1.111, p=0.748)	1.101 (0.988-1.223, p=0.078)
Injury and poisoning (S00-T98)	0.502 (0.425-0.59, p<0.001)	0.79 (0.632-0.977, p=0.034)
Other (D10-E09, E15-H95, L00-N16, N20-R99)	0.544 (0.425-0.687, p<0.001)	0.998 (0.801-1.229, p=0.983)

In females, the top 3 SMR (CI95%, ICD10 codes) for subcategories of death were pulmonary oedema 4.866 (3.815-6.122, p<0.001, J81), chronic lower respiratory 0.411 (0.305-0.542, p<0.001, J40-J47), and paralytic ileus and intestinal obstruction 1.879 (1.282-2.663, p<0.001, K56), while in males chronic lower respiratory 0.245 (0.189-0.313, p<0.001, J40-J47), tuberculosis 0.265 (0.151-0.434, p<0.001, A15-A19), and pulmonary oedema 2.979 (2.206-3.939, p<0.001, J81).

**Conclusion:** There was a significant effect of sex on standardised mortality ratios. With few exceptions, in T2DM patients oral-treated at baseline specific mortality rates generally designated as "other causes" were either similar or lower compared with the age and sex matched general population.

## 343

### HbA<sub>1c</sub> levels and all-cause mortality in type 2 diabetic patients: an epidemiological contribution to the definition of therapeutic targets

I. Dicembrini<sup>1</sup>, M. Monami<sup>2</sup>, V. Vitale<sup>3</sup>, C. Lamanna<sup>3</sup>, N. Bartoli<sup>2</sup>, D. Martelli<sup>2</sup>, S. Zannoni<sup>2</sup>, A. Antenore<sup>2</sup>, G. Toffanello<sup>2</sup>, N. Marchionni<sup>2</sup>, E. Mannucci<sup>2</sup>;

<sup>1</sup>Pathophysiological Department, Section of Endocrinology, Florence, <sup>2</sup>Department of Cardiovascular Medicine, Section of Geriatric Cardiology and Medicine, Florence, <sup>3</sup>Diabetes Agency, Careggi Teaching Hospital, Florence, Italy.

**Background and aims:** Aim of the present case-control study is to explore the effect of case mix on the relationship between HbA<sub>1c</sub> and mortality in type 2 diabetic patients (T2DM).

**Materials and methods:** A nested case-control study dataset was generated from the cohort-study dataset (n=4,140 T2DM outpatients) by sampling controls from the risk sets. Cases (N=427), were compared with an equal number (N=427) of controls chosen from those members of the cohort who were at risk for the same follow-up time of the case, matched for age (± 3 years)-, sex-, BMI (± 2 Kg/m<sup>2</sup>)-, duration of diabetes (± 5 years)-, and Charlson's Comorbidity Score (CCS) (± 1). The main predefined analysis was the comparison of cases and controls for proportion of patients with each HbA<sub>1c</sub> class (<6.5, 6.5-7.4, 7.5-8.4, and ≥8.5%) compared with Chi-square test. Chi-square test was used for between-group comparisons of categorical variables.

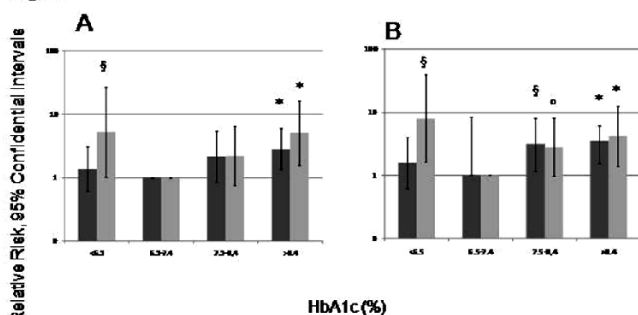
**Results:** During a mean follow-up of 5.7±3.5 years, 427 deaths were recorded. The lowest risk of death was observed in the HbA<sub>1c</sub> 6.5-7.4% category. The risk associated with a low (<6.5%) baseline and mean HbA<sub>1c</sub> was signifi-



cantly greater in patients who were insulin-treated at baseline than in the rest of the sample. A moderately elevated mean, but not baseline, HbA1c (7.5–8.4%) was associated with increased risk in non-insulin-treated patients only, whereas higher ( $\geq 8.5\%$ ) HbA1c levels, either at baseline or during follow-up, were associated with increased risk both in insulin-treated and non-insulin-treated individuals (Fig. 1, panel A and B). In a multivariate analysis, adjusting for metformin exposure and renal insufficiency, mean HbA1c during follow-up  $<6.5\%$  was associated with increased mortality in insulin-treated patients (6.22[1.06–39.82],  $p=0.045$ ), but not in non-insulin-treated subjects (1.32[0.58–3.03],  $p=0.51$ ). The increase in risk associated with HbA1c  $<6.5\%$  among insulin-treated patients was similar in those with or without known ischemic heart disease (1.64[0.90–3.80],  $p=0.25$ , and 1.72[0.90–3.29],  $p=0.10$ , respectively).

**Conclusion:** The present study suggests that glycemic targets should be individualized on the basis of the characteristics of each patient, considering age, comorbidity, and duration of diabetes. Caution should be used in prescribing insulin to reach near-normoglycemia, particularly in older, frail patients. Figure 1. Risk ratio for all-cause mortality across baseline (panel A) and mean HbA1c (panel B) categories in different subgroups of patients. Non insulin-treated patients (dark grey), insulin-treated patients (light grey). The scale is logarithmic. \* =  $p<0.01$ ;  $\dagger$  =  $p<0.05$ ;  $\circ$  =  $p<0.10$  vs reference at conditional logistic regression.

**Figure 1**



### 344

#### Total mortality by elevated transferrin saturation and hemochromatosis genotype in individuals with diabetes - risk of premature death

C. Ellervik<sup>1</sup>, H.U. Andersen<sup>2</sup>, A. Tybjaerg-Hansen<sup>1</sup>, M. Frandsen<sup>2</sup>, H. Birgens<sup>1</sup>, B.G. Nordestgaard<sup>1</sup>, T. Mandrup-Poulsen<sup>1</sup>

<sup>1</sup>Faculty of Health Sciences, University of Copenhagen, <sup>2</sup>Steno Diabetes Center, Copenhagen, Denmark.

**Background and aims:** Mortality is increased in patients with clinically overt hereditary hemochromatosis, but also in the general population with increasing transferrin saturation independently of hemochromatosis genotype. Whether increased transferrin saturation (TS) as a proxy for iron overload or hemochromatosis genotype associate with increased mortality in patients with diabetes is unknown.

**Materials and methods:** In a prospective observational study, we examined mortality according to baseline TS or hemochromatosis genotype in patients with type 1 diabetes with onset  $\geq 30$  years of age (median age: 58 years, diabetes-duration (median): 20 years) recruited in 1999 (N=716, cohort I), and mortality according to baseline TS in patients with type 1 (N=2656, age (median): 45 years, diabetes-duration (median): 26 years) or type 2 (N=3176, age (median): 63 years, diabetes-duration (median): 10 years) or other (N=288) diabetes diagnosed at any age and recruited between 2001–2007 (N=6120, cohort II). During 12 years of follow-up, 1553 patients with diabetes died.

**Results:** In cohort I+II (N=6836), the cumulative survival was decreased in patients with TS  $\geq 50\%$  versus  $<50\%$  (log-rank  $P=0.004$ , adjusted hazard ratio (HR) 1.4 (95% confidence interval (CI): 1.1–1.9;  $P=0.005$ ), population attributable risk (PAR) 2%, and median survival 75 years in TS  $\geq 50\%$  versus 80 years in TS  $<50\%$ ). Results were similar in men, in late-onset type 1 diabetes (cohort I), in any type 1 diabetes, and onset at age  $\geq 30$  years, respectively. Results were not significant in women, in cohort II, in type 2 diabetes, other diabetes, or onset at age  $<30$  years, respectively. In cohort I, cumulative survival was decreased in those with genotype C282Y/C282Y versus wild type/wild type in men (log-rank  $P=0.0001$ , adjusted HR 10.0 (3.5–28.1;  $P<0.001$ ), PAR 13%, and median survival 56 years in C282Y/C282Y versus 79 years in

wild type/wild type). In cohort I, cumulative survival for the combined exposure (TS  $\geq 50\%$  plus C282Y/C282Y) versus (TS  $<50\%$  plus wild type/wild type) in men was decreased (log-rank  $P<0.0001$ , adjusted HR 10.4 (3.7–29.3;  $P<0.001$ ), PAR 13%, and median survival 56 years in (TS  $\geq 50\%$  plus C282Y/C282Y) versus 79 years in (TS  $<50\%$  plus wild type/wild type)). No women with C282Y/C282Y died during follow-up.

**Conclusion:** Patients with diabetes with increased TS and/or hemochromatosis genotype have an increased risk of premature death, but most so in men with a 10-fold risk and with 23 years reduced life span. Two to thirteen percent of premature death among diabetics can be avoided with early screening for TS and hemochromatosis genotype.

### 345

#### Serum proenkephalin A and mortality in patients with type 2 diabetes (ZODIAC-32)

J.F.H. Arnold<sup>1</sup>, K.J.J. van Hateren<sup>1</sup>, K.H. Groenier<sup>1,2</sup>, G.J. Navis<sup>3</sup>, J. Struck<sup>4</sup>, S.J.L. Bakker<sup>3</sup>, N. Kleefstra<sup>1,3</sup>, H.J.G. Bilo<sup>1,3</sup>

<sup>1</sup>Diabetes Centre, Isala Clinics, Zwolle, Netherlands, <sup>2</sup>General Practice, University Medical Center Groningen, Netherlands, <sup>3</sup>Internal Medicine, University Medical Center Groningen, Netherlands, <sup>4</sup>Research Department, B.R.A.H.M.S GmbH (Thermo Fisher Scientific), Hennigsdorf/Berlin, Germany.

**Background and aims:** Enkephalins are endogenous opioid peptides derived from the precursor hormone proenkephalin A. These peptides have been found in the pancreatic islets and have been suggested to play a role in glucose metabolism. In addition, opioid peptides are also involved in a variety of cardiovascular pathways, and can e.g. influence blood pressure. Therefore, we aimed to investigate whether Proenkephalin A (PENK-A) was related to all-cause and cardiovascular mortality in patients with type 2 diabetes mellitus (T2DM).

**Materials and methods:** This study is part of the ZODIAC study, a prospective observational study of primary care patients with T2DM, and incorporates two cohorts (1998 and 2001) with a number of 1689 patients. EDTA plasma was stored at  $-80^{\circ}\text{C}$ , until assessment of PENK-A by sandwich immunoassay. Data on mortality were collected in 2009. Primary endpoints were all-cause and cardiovascular mortality. Cox-proportional hazards models were used to investigate the relationship between PENK-A and mortality with adjustment for selected confounders. Three models were chosen: model 1 included only PENK-A, model 2 included age and sex and model 3 included 9 cardiovascular risk factors as additional potential confounders. Harrell C statistic was used to investigate the capability of each model to predict mortality. PENK-A was logarithmically transformed because of skewed distribution of the data.

**Results:** PENK-A was assessed in baseline serum samples of 1204 patients; age  $66.8 \pm 11.6$  years, 45.2% males, with a median [interquartile range] PENK-A concentration of 112.0 [91.2–143.0] pmol/l. Other baseline data include mean HbA1c of  $55.6 \pm 9.0$  mmol/mol and a median diabetes duration of 4 [2–9] years. During a median follow-up period of 5.5 [3.1–10.1] years, 361 (30.0%) patients had died of which 153 (42.4%) were attributed to a cardiovascular cause. Table 1 presents the hazard ratios (HR) of log PENK-A for mortality and the Harrell C's for the three models, showing an increased HR for mortality for the crude model, and after adjustment for age and gender. However, in model 3, the association of log PENK-A with mortality lost its significance. According to the Harrell C statistic, model 3 is the most capable for predicting mortality. Removing PENK-A from model 2 and 3 yielded no significant change in the prediction of mortality and no relevant change in Harrell's C values.

Table 1:

Primary endpoints	Model 1	Model 2	Model 3
<b>All-cause mortality</b>			
log PENK-A: HR (95% CI)	3.78 (2.80–5.11)	1.54 (1.11–2.13)	1.31 (0.91–1.89)
Harrell's C (95% CI)	0.63 (0.59–0.66)	0.77 (0.75–0.80)	0.80 (0.78–0.82)
<b>Cardiovascular mortality</b>			
Log PENK-A: HR (95% CI)	5.51 (3.49–8.69)	2.70 (1.62–4.51)	1.61 (0.90–2.87)
Harrell's C (95% CI)	0.65 (0.60–0.70)	0.75 (0.71–0.80)	0.81 (0.78–0.85)

**Conclusion:** Increased levels of PENK-A were associated with an increased risk of all-cause and cardiovascular mortality in type 2 diabetes, even after adjusting for gender and sex. However, after adjustment for several cardiovascular risk factors this significant association was no longer observed.

## PS 010 Burden of diabetes and approaches to decrease it

346

### Trends in epidemiology and costs of diabetes in Italy. The ARNO observatory database

R. Miccoli, On behalf of the SID-CINECA “ARNO-Diabetes” Working Group;

Department of Endocrinology and Metabolism, Pisa University, Pisa, Italy.

**Background:** Accurate data about the magnitude and distribution of diabetes mellitus can inform policy and support health care evaluation. By using physician prescriptions and other administrative data we aimed to describe trends in incidence and prevalence of diabetes in Italy over time.

**Materials and methods:** The ARNO-Diabetes is a data base comprising 9,465,492 citizens resident in 29 Local Health Districts (LHD) during 2010 and a large cohort of 2,135,494 living in 10 LHD with data spanning from 1997 to 2010. Three administrative databases with record linkage were used in the present analysis.

**Results:** In the 2010, 544,852 patients with diabetes (49% female, F; 51% male, M), were identified by using drug prescription data (88%), hospital records (ICD-9)(15%) and exemptions from medical charge (76%). Prevalence of disease was 5.8% (F 6.1%, M 5.5%) with an increasing gradient from northern to southern Italian areas. Nearly two third of cases were aged over 64, with one third being over 80. Only 0.5 and 2% of cases were below 19 and 34, respectively. Twelve percent of patients were on diet only, 67% were being treated with oral antidiabetic drugs (OAD), 10% with OAD+insulin, 11% with insulin alone. The prevalence of diabetes increased steadily from 2.8% in 1997, particularly in M (+87% vs. +52% in F) with an annual incidence of 7 cases per 1000 persons-year. Drug treatment represented only 1/3 of total cost attributed to diabetes, with an increase of 132% in the same period. A 97% increase of cost has been registered for OAD while that for insulin was 184%, respectively.

**Conclusion:** During the last 15 years a significant increase in diabetes prevalence occurred in Italy, with a corresponding rise of costs. The combined use of different administrative databases is a feasible and efficient method to determine and monitor the incidence, prevalence and costs of diabetes.

Supported by: Italian Society of Diabetology

347

### Demographic modelling of the direct medical excess costs of type 2 diabetes mellitus in Germany from 2010 to 2040

R. Waldeyer<sup>1,2</sup>, R. Brinks<sup>2</sup>, W. Rathmann<sup>2</sup>, G. Giani<sup>2</sup>, A. Icks<sup>1,2</sup>;

<sup>1</sup>Public Health Department, Faculty of Medicine, Heinrich Heine University, Düsseldorf, <sup>2</sup>Institute for Biometry and Epidemiology, German Diabetes-Center, Düsseldorf, Germany.

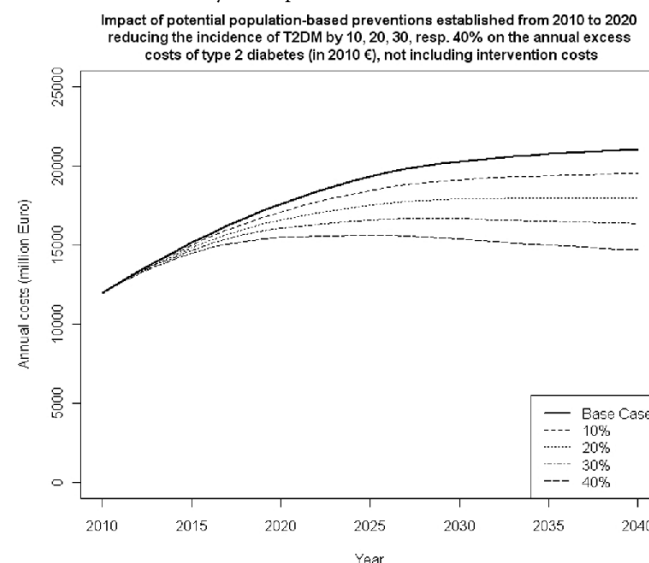
**Background and aims:** Comprehensive analyses of the future course of diabetes-related costs in Germany are lacking. We model a country-specific forecast of direct medical costs of type 2 diabetes. Demographic changes, disease dynamics and undetected cases are taken into account. Consequences of hypothetical population-based preventive interventions are analyzed.

**Materials and methods:** A time-discrete Markov model is developed to estimate stratum-specific future prevalences of diabetes (diagnosed/undetected) 2010–2040 in the population over 40 years. They are linked with cost weights. Demographic, epidemiologic, and economic scenarios are modelled. Model inputs include the official population forecasts, current prevalences, incidences, mortalities, proportions of undetected cases, per capita health expenditures of the general population, and cost ratios of a person without diabetes vs. a diabetic person (diagnosed/undetected). Model outputs are the numbers of cases and the associated annual direct medical excess costs of type 2 diabetes (in 2010€) from a societal perspective.

**Results:** In the base case, case numbers rise steeply until 2030. Then the growth levels off until the maximum of 8.0 million diseased people is reached in 2037 (of which 3.4 million are undetected). The prevalence of over 40-year-olds increases from 10.6% in 2010 to 16.1% in 2040. The numbers of cases decrease in age groups under 60 and more than double in age groups over 75 years. The annual costs increase from 2010 (11.9 billion Euros) to 2030 by 74%. The growth rate gets smaller afterwards. In 2040, costs of 21.1 billion Euros are expected (Figure). In the scenario of constant prevalences over time, costs in-

crease to 15.1 billion Euros. The results are sensitive to rising per capita costs, too. They do not vary a lot due to demographic scenarios and proportions of undetected cases. If a prevention to lower the incidence by 10%, 20%, 30%, resp. 40% would be established over the next 10 years, it would lead to cumulative savings of 25, 51, 77, resp. 105 billion Euros in diabetes-related health expenditures from 2010 to 2040 (not including intervention costs), see Figure.

**Conclusion:** The projected increase in cases over the next 30 years is due to demographic changes and disease-dynamics. It is enhanced by higher per capita costs with age, causing a stronger growth rate of the predicted costs. Better epidemiologic and economic data of diabetes care in Germany is needed to improve the forecasting accuracy. Nevertheless, the results suggest a continuing rise in diabetes-related health expenditures. The hypothetical potential of prevention is shown and needs to be further evaluated in cost-effectiveness analyses. The forecasting method can be developed further to anticipate possible impacts of alternative policy scenarios of prevention and intervention. It can easily be adapted to other chronic diseases as well.



348

### Who are caring for patients with childhood-onset adult type 1 diabetes in Japan? Paediatric or adult diabetologists? The DERI mortality study

Y. Onda<sup>1</sup>, A. Morimoto<sup>1</sup>, R. Nishimura<sup>1</sup>, H. Sano<sup>1</sup>, K. Utsunomiya<sup>1</sup>, N. Tajima<sup>2</sup>;

<sup>1</sup>Department of Internal Medicine Division of Diabetes and Endocrinology,

<sup>2</sup>The Jikei University School of medicine, Tokyo, Japan.

**Background:** The timing for transition of patients with childhood-onset type 1 diabetes (T1D) from pediatric (PED) to adult diabetology (AD) has become the subject of debate in recent years, which led to a position statement being released by the ADA transition working group, and this topic is also being hotly debated in Japan, where it is not rare that these patients continue to consult PED even after attaining adulthood.

**Objective:** To investigate the status of care for patients with T1D by their attending physicians, 25 years after diagnosis.

**Methods:** Of the 1,387 patients (556 males/831 females) registered with two nationwide T1D surveys in Japan who were diagnosed as having T1D at less than 18 years of age between 1965 and 1979, the present study included a total of 859 patients (330/529) whose attending physicians were confirmed as of January 1, 2000. Their attending physicians and affiliations were determined based on completed questionnaires. We evaluated the percentage of the attending physicians as they were accounted for by PED, AD, and other specialists (OS). We also investigated factors that contributed to these patients consulting PED by using the logistic regression model with the age at diagnosis, sex, and the calendar year of diagnosis included as predictive variables. All statistical analyses were performed by using SAS 9.2.

**Results:** The mean age at diagnosis of the 859 patients was  $8.5 \pm 4.1$  (SD) years, with a duration of diabetes  $25.2 \pm 4.0$  years. Their mean attained age as of January 1, 2000 was  $33.7 \pm 5.8$  years. Of these, 16.4% (141 patients, 57 males/84 females) consulted PED, 81.3% (698, 266/432) consulted AD, and 2.3% (20, 7/13) consulted OS. By their age at diagnosis, of the 667 patients

diagnosed at less than 12 years old, 19.5% consulted PED, 78.7% AD, and 1.8% consulted OS, while, of those diagnosed at 12 years old or older (192 patients), 5.7% consulted PED, 90.1% AD, and 4.1% OS. Significantly more patients consulted PED among those diagnosed at less than 12 years old than among those diagnosed at 12 years old or older ( $P < 0.001$ , chi-square test). There was no significant sex difference by age of diagnosis in the percentage of those who consulted PED. By their attained age, of the 244 patients whose attained age was less than 30 years old, 29.1% consulted PED, 68.9% AD, and 2.1% OS. Of the 615 patients whose attained age was over 30 years old, 11.4% consulted PED, 86.2% AD, and 2.4% OS. Significantly more patients consulted PED among those whose attained age was less than 30 years old than among those whose attained age were over 30 years old ( $P < 0.001$ ). There was no significant sex difference by attained age in the percentage of those who consulted PED. Logistic regression analyses revealed that younger age at diagnosis and more recent year of diagnosis but not sex were significant predictors of these patients consulting PED.

**Conclusion:** Even 25 years after diagnosis, 16.4 % of Japanese patients with childhood-onset adult T1D consulted PED. Significantly more patients consulted PED among those diagnosed at less than 12 years old and those whose attained age were less than 30 years old, compared with those diagnosed at 12 years old or older and those whose attained age was over 30 years old. About 10% of patients over 30 years of age still consulted PED.

*Supported by: The Ministry of Education, Culture, Sports, Science, and Technology, Japan*

### 349

#### Health-related quality of life in type 2 diabetes patients taking oral anti-diabetic drugs in urban China

W. Jia<sup>1</sup>, Y. Shen<sup>2</sup>, J. Lu<sup>3</sup>, J. Weng<sup>4</sup>, L. Ji<sup>5</sup>, China DiaSTAGE Study Group;

<sup>1</sup>Shanghai Jiao Tong University Affiliated 6th People's Hospital, Shanghai,

<sup>2</sup>Zhejiang University School of Medicine, Hangzhou, <sup>3</sup>Chinese People's Liberation Army General Hospital, Beijing, <sup>4</sup>Sun Yat-sen University 3rd Hospital, Guangzhou, <sup>5</sup>Peking University People's Hospital, Beijing, China.

**Background and aims:** To understand the health-related quality of life (HRQoL) among type 2 diabetes (T2D) patients taking oral anti-diabetic drugs (OAD) and its associated determinants in Urban China.

**Materials and methods:** A nationwide, cross-sectional, multicenter study was conducted between Jun - Nov 2010. A total of 9872 patients with T2D were recruited from 103 centers. A standardized case report form was used by on-site physicians to collect and record patient data, including socio-demographic characteristics, disease profile and treatment pattern. Patient's HRQoL was assessed by visual analog scale (VAS) and EuroQol-5 Dimensions (EQ-5D). Logistic regressions and linear regressions were used to explore determinants of responses in the EQ-5D dimensions.

**Results:** 2805 patients had well-controlled blood-glucose ( $HbA1c < 6.5$ ), accounting for 28.63% of the study population. Average VAS and EQ-5D score were  $78.59 \pm 11.45$  and  $0.810 \pm 0.081$ , respectively. The percentages of patient reporting "no problem" in five dimensions were high in the middle and low in both ends along the corresponding HbA1c level ( $< 6.5$ ,  $6.5-7.5$ ,  $> 7.5$ ). The probability of reporting "problems" in Mobility dimension was 1.72-fold ( $P = 0.0091$ ) in blood-glucose uncontrolled group compared to that in well-controlled group. VAS and EQ-5D scores were lower in uncontrolled group ( $78.07 \pm 11.45$ ,  $0.807 \pm 0.083$ ,  $P < 0.0001$ ) than in well-controlled group ( $79.82 \pm 11.30$ ,  $0.817 \pm 0.074$ ,  $P < 0.0001$ ). Course of disease was weakly correlated with VAS and EQ-5D scores, with negative correlation coefficient of  $-0.04$  ( $P = 0.0003$ ) and  $-0.13$  ( $P < 0.0001$ ). Hypoglycemia increased the probability of reporting "problems" in 4 dimensions except for Mobility. Patients with hypoglycemia had a lower EQ-5D score compared to patients without hypoglycemia ( $0.792 \pm 0.098$  VS  $0.813 \pm 0.078$ ,  $P < 0.0001$ ), but found no such impact on VAS scores. Average BMI in T2D patients was  $24.50 \pm 2.96$ . BMI was negatively associated with the probability of reporting "problems" in Anxiety/Depression dimension ( $P = 0.0007$ ) but was positively correlated with VAS and EQ-5D scores ( $P < 0.0001$ ,  $P < 0.0072$ , respectively). Complications increased the probability of reporting "problems" in all five dimensions ( $P < 0.0001$ ). The strongest determinants of increased problem reporting were stroke and diabetic foot. VAS and EQ-5D scores were lower in patients with complications ( $78.40 \pm 11.57$ ,  $0.807 \pm 0.084$ ) compared to patients without ( $81.42 \pm 9.73$ ,  $0.832 \pm 0.047$ ) ( $P < 0.0001$ ).

**Conclusion:** Well-controlled blood-glucose is associated with improved HRQoL in T2D patients while hypoglycemia, lower BMI and complications may have substantial negative impact on several dimensions of HRQoL.

Table 1. EQ-5 Dimensions and VAS scores at different HbA1c level

Dimensions	Statements	HbA1c(%)			X <sup>2</sup>	P value
		<6.5	6.5-7.5	>7.5		
Mobility	No problems	2239 (32.91)	2836 (40.54)	1920 (27.45)	46.7583	<0.0001
	Some problems	58 (15.80)	186 (40.17)	192 (41.65)		
	Extreme problems	9 (32.14)	8 (28.57)	11 (39.29)		
Self-care	No problems	2290 (31.76)	2922 (40.35)	1994 (27.67)	37.5261	<0.0001
	Some problems	34 (13.33)	102 (10.00)	119 (46.67)		
	Extreme problems	12 (10.00)	8 (26.67)	10 (33.33)		
Usual activities	No problems	2245 (31.99)	2955 (40.36)	1939 (27.55)	37.9255	<0.0001
	Some problems	83 (19.26)	170 (39.44)	178 (41.30)		
	Extreme problems	8 (36.10)	7 (33.33)	6 (28.57)		
Pain/discomfort	No problems	1949 (32.05)	2474 (40.58)	1659 (27.23)	20.3140	<0.0001
	Some problems	377 (27.42)	546 (39.85)	450 (32.78)		
	Extreme problems	10 (29.41)	10 (29.41)	14 (41.18)		
Anxiety/Depression	No problems	2023 (32.35)	2561 (40.36)	1686 (26.69)	56.4941	<0.0001
	Some problems	308 (26.65)	460 (38.30)	433 (38.05)		
	Extreme problems	5 (13.51)	11 (26.73)	21 (56.76)		
EQ-VAS score		$80.19 \pm 11.34$	$79.24 \pm 11.19$	$76.90 \pm 12.13$	103.2880	<0.0001
EQ-5D score		$0.818 \pm 0.073$	$0.812 \pm 0.077$	$0.796 \pm 0.083$	64.3248	<0.0001

*Supported by: Beijing Novartis Pharma Co., Ltd.*

### 350

#### Determinants of lifestyle behaviour modification in type 2 diabetes: the survey on living with chronic diseases in Canada

J.A. Johnson<sup>1</sup>, C.B. Agborsangaya<sup>1</sup>, M.E. Gee<sup>2</sup>, S.T. Johnson<sup>3</sup>, P. Dunbar<sup>4</sup>, M.-F. Langlois<sup>5</sup>, L. Leiter<sup>6</sup>, C. Pelletier<sup>2</sup>;

<sup>1</sup>Department Of Public Health Sciences, University of Alberta, Edmonton,

<sup>2</sup>Public Health Agency of Canada, Ottawa, <sup>3</sup>Faculty of Health Disciplines,

Athabasca University, Athabasca, <sup>4</sup>Diabetes Care Program of Nova Scotia,

Halifax, <sup>5</sup>Division of Endocrinology, Centre Hospitalier Universitaire de

Sherbrooke, <sup>6</sup>Division of Endocrinology & Metabolism, Keenan Research

Centre in the Li Ka Shing Knowledge Institute of St. Michael's Hospital, Toronto, Canada.

**Background and aims:** Lifestyle modification is an important cornerstone for the management of type 2 diabetes (T2D). We determined the prevalence of engagement in lifestyle behavior change, as well as the impact of healthcare professional support on these behaviors using national-level data.

**Materials and methods:** Self-reported data was available from 2682 adult patients with T2D, age 20 years or older, who responded to the 2011 Survey on Living with Chronic Diseases in Canada's diabetes component. Multivariate prevalence rate ratios (RR) for never engaging in, and not sustaining self-management behaviors (of dietary change, weight control, exercise, and smoking cessation) were evaluated using binomial regression models.

**Results:** The prevalence of reported dietary change, weight control/loss, increased exercise and smoking cessation (among those who smoked since being diagnosed), were 89.7%, 72.1%, 69.5%, and 30.6%, respectively. Participants who reported not receiving health professional advice were more likely to report never engaging in dietary change (RR = 2.7, 95% CI 1.8 - 4.2), exercise (RR = 1.7, 95% CI 1.3 - 2.1), or weight control/loss (RR = 2.2, 95% CI 1.3 - 3.6), but not smoking cessation (RR=1.0, 95% CI 0.7, 1.5). Also, living with diabetes for more than six years was associated with not sustaining dietary change, weight loss and smoking cessation, but not exercise.

**Conclusion:** Health professional advice may support individual action for lifestyle behaviors for T2DM self-management. Patients living with the disease for more than 6 years may require additional support in sustaining recommended behaviors.

*Supported by: CIHR Team Grant to ACHORD (ref#: OTG-88588)*

### 351

#### Response to tailored therapy in a heterogeneous type 2 diabetes mellitus population: simulation results from a qualified mathematical model

A. De Gaetano<sup>1</sup>, S. Panunzi<sup>1</sup>, T.A. Hardy<sup>2</sup>;

<sup>1</sup>Biomathematics Lab, Cnr Iasi, Rome, Italy, <sup>2</sup>Eli Lilly & Company,

Indianapolis, USA.

**Background and aims:** Disease heterogeneity in T2DM is manifest in multiple ways, including age of onset, rate of disease progression, risk of complications, and response to available therapies. Tailored therapeutics (TTx) involves matching treatment options to individual patient characteristics with the goal of maximizing the benefit/risk ratio. In the current work, we have used a mechanistic mathematical model of T2DM long-term evolution (Diabetes Progression Model, DPM) to explore TTx hypotheses. DPM has previously been shown to faithfully simulate the natural progression of T2DM over several years, and to recapitulate the effects of various interventions represented in the Diabetes Progression Program and CANOE studies.



**Materials and methods:** The evolution of fasting glycemia, fasting insulinemia and beta-cell mass were simulated for virtual patients with a range of defects in insulin secretion, insulin sensitivity, or both. For each hypothesized time course of the primary defects or their combination, the natural history of the disease as well as the response to several interventions or sequences of interventions were simulated. Among the therapeutic approaches tested are pharmacologic interventions directed at improving insulin sensitivity and insulin secretion proportionally to underlying metabolic status and secretory ability, as well as radical maneuvers hypothetically restoring a fraction of normal sensitivity or secretion, such as metabolic/bariatric surgery or successful elimination of inflammation-induced inhibition of beta-cell replication. Results were compared to available published information.

**Results:** Response to individual therapies is influenced not only by the degree of insulin resistance or insulin secretory defect, which is a function of the type and stage of progression of the original abnormality, but also by the accumulated injury to beta-cell replicating ability, which depends on the duration and severity of the primary defect. Therapy directed at proportional improvement of the current insulin sensitivity or secretion levels has largely equivalent effects, independent of the nature of the original defect. This effect lacks durability if started after development of hyperglycemia. Durable results can be obtained with drastic and permanent lifestyle changes or by administering conventional therapy as soon as reasonable suspicion of deteriorating insulin sensitivity or secretion is entertained.

**Conclusion:** Tailored therapeutic approaches can be explored prospectively, for a given patient, by the use of diabetes progression model simulations, detailing response to interventions based on therapeutic mechanism and underlying pathophysiology. For patients with advanced degrees of insulin sensitivity or secretion defects, simulations are consistent with clinical observation in predicting short-lived efficacy of conventional pharmacologic treatment. More articulate TTx schemes can however be designed, their likely efficacy evaluated over a realistically heterogeneous population of virtual subjects, and promising approaches, with improved benefit/risk ratios, can then be tested in real-world clinical trials.

interventional programme was 686€ compared to 646€ in the non intervention group. The incremental cost of the individual and group intervention compared to the standard preventive care was 10€ and 106€ and the incremental cost-effectiveness ratio (ICER) was 746€ and 108€ per averted case of diabetes, respectively. The QoL (utility) at the end of study was higher for the intervention group (0.93 vs. 0.91) when compared to the control cohort ( $p=0.01$ ), being the incremental cost-utility ratio 3243 €/QALY gained. At the sensitivity analysis, costs were very sensible to different sources of costing though remaining at an acceptable level. Limitations of the study include the variability of healthcare resource usage and not having included the induced resource usage.

**Conclusion:** Taking into account the costs of managing patients with diabetes, this study shows that a PHC led intensive lifestyle intervention among high-risk people is not only effective but also efficient in delaying progression to diabetes. The future implementation of this model could be highly recommended.

*Clinical Trial Registration Number: NCT01519505*

*Supported by: SANCO grant 2004310, FIS PI05-033 and PS09-001112; Department of Health*

## 352

### Efficiency of a community based intervention on high risk individuals for developing diabetes. The PREDICE & DE-PLAN-CAT study

J.J. Cabre<sup>1</sup>, O. Solà-Morales<sup>2</sup>, B. Costa<sup>1</sup>, B. Sunyer<sup>3</sup>, R. Sagarra<sup>1</sup>, F. Barrio<sup>1</sup>, B. Bolibar<sup>1</sup>, C. Castell<sup>4</sup>;

<sup>1</sup>Diabetes & Metabolism, Jordi Gol Primary Care Research Institute, Reus-Barcelona, <sup>2</sup>Chief Medical Director, SabirMedical, Barcelona, <sup>3</sup>Catalan Agency for Health Information, Assessment and Quality, Barcelona,

<sup>4</sup>Department of Health, Barcelona, Spain.

**Background and aims:** One of the main tasks of primary health care (PHC) is to implement strategies leading to improve efficiently the care of the population. It is well known that individuals at high risk, particularly those diagnosed as having prediabetes, tend to progress to Type 2 diabetes. We have previously shown that a PHC lifestyle intervention is effective in delaying progression to diabetes among high-risk individuals. The objective of this study was to show that the strategy is also efficient.

**Materials and methods:** Data on resource utilisation and quality of life (QoL) were collected alongside a prospective cohort study conducted in 18 PHC centres evaluating the effectiveness of an intensive lifestyle intervention. People were eligible for the lifestyle intervention only if they had had an oral glucose tolerance test, did not have diabetes and had either or both of a FINDRISC score  $\geq 14$  or prediabetes (WHO criteria for fasting or 2 h glucose). Where feasible, high-risk identified individuals were allocated sequentially to standard care, a group-based or an individual level intervention (intensive reinforced DE-PLAN intervention). Details of the individual resources used (physician and nursing time, number of visits...) were collected during the trial on a random subsample of the cohort. Average costs were calculated using the apportioned costing data recorded. The incidence of diabetes was considered the primary effectiveness outcome but also QoL which was captured periodically using the 15D questionnaire till participants developed diabetes. The results were converted into utility measures. Costs and QoL differences were analysed and a cost-effectiveness analysis was then performed.

**Results:** The incidences of diabetes after 4.2 years median follow-up were 4 and 3.6 cases per 100 person-years for both group ( $n=230$ ) and individual ( $n=103$ ) intensive interventions and 7.2 per 100 person-years in the standard-care ( $n=219$ ) control group. The cumulative incidence after 4 years follow-up was 20% and 14.6% for both group and individual intervention and 28.8% in the standard care group. The average costs per person of the

## PS 011 Characterisation of patients with type 2 diabetes

353

### Diabetes prevalence among 2.4 million Health Maintenance Organisation (HMO) members < 20 years of age, 2005–2009: the SUPREME-DM project

J.M. Lawrence<sup>1</sup>, P.J. O'Connor<sup>2</sup>, J.R. Desai<sup>3</sup>, R.J. Reid<sup>3</sup>, E. Schroeder<sup>4</sup>, S. Vupputuri<sup>5</sup>, X.S. Yan<sup>6</sup>, J.F. Steiner<sup>4</sup>, G.A. Nichols<sup>7</sup>, for the SUPREME-DM Study Group;

<sup>1</sup>Kaiser Permanente Southern California, Pasadena, <sup>2</sup>HealthPartners Research Foundation, Minneapolis, <sup>3</sup>Group Health Cooperative, Seattle, <sup>4</sup>Kaiser Permanente Colorado, Denver, <sup>5</sup>Kaiser Permanente Southeast, Atlanta, <sup>6</sup>Geisinger Health System, Danville, <sup>7</sup>Kaiser Permanente Northwest, Portland, USA.

**Background and aims:** Despite the growing concern about the incidence and prevalence of diabetes mellitus (DM) among youth, few data sources allow for the tracking of U.S. cases on a population level. Electronic health records (EHR) provide an opportunity to identify persons with DM in large diverse populations. We estimated annual DM prevalence by age category and sex over 5 years, 2005–2009, among members of 11 HMOs from 10 U.S. States who were <20 years of age.

**Materials and methods:** For each year, the denominator was composed of health plan members < 20 years of age on the last day of the year with ≥ 6 months of health plan membership. The numerator included individuals with ≥ 2 laboratory test results indicative of DM, ≥ 1 inpatient or ≥ 2 outpatient DM diagnoses, or dispensed any glucose-lowering medication other than metformin, outside of any pregnancy period. We estimated DM prevalence/1,000 members by age and sex, and calculated absolute rate change and % change from 2005 to 2009. We report glucose-lowering medication use for youth ≥ 10 years, categorized as insulin only (probable type 1), oral agent ± insulin (probable type 2), and no medications (probable type 2 or undiagnosed DM).

**Results:** The average annual denominator was 2,406,796 HMO members < 20 years of age. DM prevalence increased by 9.1%, from 2.70/1,000 in 2005 to 2.95/1,000 in 2009 (Table). Increases were observed among youth 5–9 years (15.6%), 10–14 years (8.2%) and 15–19 years (11.8%) with a 19.4% decline among youth < 5 years. Among 10–14 year olds, increases were seen in the oral agent ± insulin group and among those not using medications. Among 15–19 year olds, increases were seen in all three treatment groups, with the largest absolute and relative increases among insulin users. DM prevalence was higher for girls than boys for all years (e.g., 3.00/1,000 for girls vs. 2.77/1,000 for boys in 2009). Among youth ages 10 and older, girls with DM were more likely than boys to be in the insulin only group.

**Conclusion:** Using EHR data from members of 11 geographically dispersed HMOs; we found that the prevalence of DM was higher than previously reported for U.S. youth. This may be due to differences in the populations under surveillance, approaches to case ascertainment and definition, the inclusion of undiagnosed cases, or an increase in DM prevalence in recent years. Prevalence was higher for girls than for boys, a finding observed in other studies. The rise in probable type 2 DM prevalence among 10–14 year-olds is of particular concern. EMRs provide a cost-effective opportunity to provide sustainable nation-wide childhood diabetes surveillance.

Age, Years	Mean Annual Denominator/ Medication Category	2005	2006	2007	2008	2009	Absolute 5-Year Change	% 5 Year Change
0–4	470,921	0.77	0.67	0.65	0.59	0.62	-0.15	-19.4%
5–9	576,750	1.49	1.56	1.59	1.65	1.72	0.23	15.6%
10–14	654,471	2.98	3.00	3.06	3.07	3.22	0.24	8.2%
	Insulin only	2.06	2.06	2.09	2.01	2.01	-0.05	-2.5%
	Oral agent ± insulin	0.26	0.27	0.28	0.28	0.35	0.09	35.1%
	No medications	0.66	0.67	0.69	0.78	0.86	0.20	31.1%
15–19	704,654	4.73	4.79	5.09	5.17	5.29	0.56	11.8%
	Insulin only	2.52	2.51	2.77	2.80	2.86	0.34	13.5%
	Oral agent ± insulin	0.88	0.96	0.96	0.93	0.97	0.09	10.3%
	No medications	1.33	1.33	1.35	1.44	1.46	0.13	9.6%
Total	2,406,796	2.70	2.73	2.83	2.86	2.95	0.25	9.1%

Supported by: AHRQ R01HS019859

354

### Long-term patterns of HbA<sub>1c</sub> among patients with type 2 diabetes mellitus

E.J.F. Lamberts<sup>1</sup>, M.L. Bouvy<sup>1</sup>, P.C. Souverein<sup>1</sup>, G. Nijpels<sup>2</sup>, L.M.C. Welschen<sup>3</sup>, J.M. Dekker<sup>2</sup>, J.G. Hugtenburg<sup>3</sup>, E. van 't Riet<sup>2</sup>;

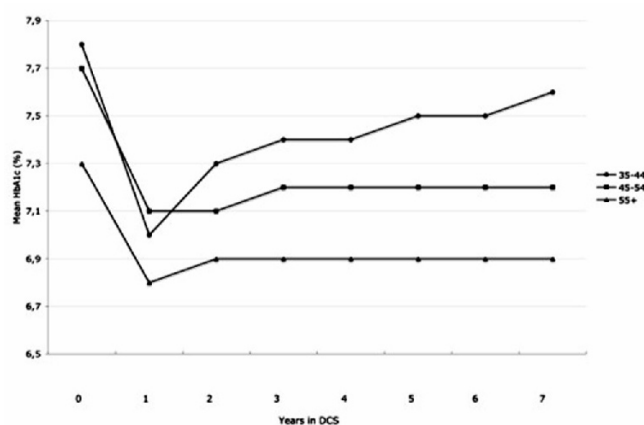
<sup>1</sup>Division of Pharmacoepidemiology and Pharmacotherapy, Utrecht Institute for Pharmaceutical Sciences, <sup>2</sup>Department of General Practice and the EMGO Institute for Health and Care Research, Amsterdam, <sup>3</sup>Department of Clinical Pharmacology and Pharmacy, Amsterdam, Netherlands.

**Background and aims:** Treatment guidelines recommend in general strict glycemic control. HbA<sub>1c</sub> (glycosylated hemoglobin) has been used as a marker for glycemic control. Maintaining appropriate HbA<sub>1c</sub> levels and lowering cardiovascular risk factors have proven to decrease micro vascular and macro vascular disease. Identifying long-term trends and predictors of glycemic control may enable clinicians to identify patients at risk of inadequate glycemic control. The objective of this study was to determine longitudinal HbA<sub>1c</sub> categories in daily clinical practice of patients with type 2 diabetes and to identify predictors of these categories.

**Methods:** A retrospective observational cohort study was conducted among patients with type 2 diabetes. Data were obtained from a protocolled Diabetes Care System (DCS), situated in West-Friesland, The Netherlands, a region that has approximately 200,000 inhabitants and is representative for a Dutch and Western-European population. All annually measured clinical data, including HbA<sub>1c</sub>, were registered in a central database. For each patient, linear regression analysis was conducted starting from the second HbA<sub>1c</sub> measurement to the end of follow-up. The slope of the regression line ( $\beta$ -coefficient) was used as an indicator for the individual time trend of the HbA<sub>1c</sub> progress. Patients were classified as either deteriorating ( $\beta$ -coefficient > +0.1), improving ( $\beta$ -coefficient < -0.1) or stable ( $\beta$ -coefficient between -0.1 and +0.1). Logistic regression analysis was used to calculate the odds ratios of the potential predictors for HbA<sub>1c</sub> deterioration with combined improving and stable categories as reference category with 95% confidence intervals. Crude and adjusted odds ratios were calculated. Adjustment was done for all the predictors in the total model. We stratified for age category at baseline to assess HbA<sub>1c</sub> progress up to seven years of treatment.

**Results:** We included 4,689 patients in the study cohort. HbA<sub>1c</sub> levels in the youngest group (35–45 years) increased more strongly compared to the older age groups who tended to stabilize at sub clinical levels of 7.5% (58 mmol/mol), while the 45–54 age category stabilizes at 7.2% (55 mmol/mol). Younger age was associated with an increased risk of deterioration compared to older age (adjusted odds ratio 1.44 (95% CI: 1.04–1.99)).

**Conclusion:** Patients with younger age at baseline are more likely to be in the deteriorating HbA<sub>1c</sub> category. Our findings recommend more intensive monitoring and treatment of younger patients. More research is needed for a better understanding of the relationship between younger age and long term increase in HbA<sub>1c</sub>.



Mean HbA<sub>1c</sub> by age category shown per year of treatment in the Diabetes Care System (DCS)

## 355

**Relative contribution of insulin resistance and insulin deficiency in the development of type 2 diabetes in Koreans**C.-H. Kim<sup>1</sup>, H.-K. Kim<sup>2</sup>, E.-H. Kim<sup>2</sup>, S.-J. Bae<sup>2</sup>, J.-Y. Park<sup>3</sup><sup>1</sup>Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, Kyeonggi-do, <sup>2</sup>Health Screening & Promotion Center, Asan Medical Center, Seoul, <sup>3</sup>Internal Medicine, University of Ulsan College of Medicine, Seoul, Republic of Korea.

**Background and aims:** Type 2 diabetes is a heterogeneous disorder characterized by varying degrees of insulin resistance and relative insulin deficiency. However, it is still unclear whether the relative contribution of insulin resistance and beta-cell dysfunction in the pathogenesis of type 2 diabetes differs by ethnicity. We assessed indices of insulin resistance and beta-cell function in non-diabetic Korean individuals and examined their association with later development of type 2 diabetes.

**Materials and methods:** We analyzed the clinical and laboratory data of 17,878 Korean adults (age 20–79 years, 11,218 men and 6,660 women) who underwent routine medical check-ups in 2007–08 (baseline) and again in 2010–11 (follow up) with a mean 3.5-year (range 2.5–4.5 years) interval. All non-pregnant adults who did not have diabetes at baseline examination were included for the analysis. Insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B) indices at baseline were assessed by homeostasis model assessment. The presence of insulin resistance and beta-cell dysfunction were defined as HOMA-IR  $\geq 75$  percentile and HOMA-%B  $\leq 25$  percentile values of non-obese participants who had normal fasting glucose levels and no metabolic syndrome ( $n=9525$ ). Odds ratios (ORs) for incident type 2 diabetes were estimated using multiple logistic regression analysis.

**Results:** Among the 17,878 participants who did not have diabetes at baseline, a total of 732 subjects (4.1%, 571 men and 161 women) developed type 2 diabetes during the 3.5-year follow-up period. Those who developed type 2 diabetes had significantly higher fasting serum insulin level ( $8.9 \pm 5.2$  vs.  $6.9 \pm 3.9$  mU/l,  $P < 0.001$ ) and HOMA-IR ( $2.38 \pm 1.45$  vs.  $1.65 \pm 1.02$ ,  $P < 0.001$ ), and low HOMA-%B ( $74 \pm 47$  vs.  $85 \pm 48$ ,  $P < 0.001$ ) at baseline. The cut-off values for insulin resistance and beta-cell dysfunction were HOMA-IR  $\geq 1.72$  (75th centile) and HOMA-%B  $\leq 53$  (25th centile), respectively. Among the subjects who developed type 2 diabetes, 29% showed predominant beta-cell dysfunction, 51% had predominant insulin resistance, and 10% had combined defects. When we divided the participants according to the median BMI of the whole population at baseline ( $23.7 \text{ kg/m}^2$ ), 49% had predominant beta-cell dysfunction and 26% had predominant insulin resistance in lower BMI group, while 21% had mainly beta-cell dysfunction and 60% had mainly insulin resistance in the higher BMI group. The odds ratio of incident type 2 diabetes for the insulin resistance (HOMA-IR  $\geq 75$  percentile vs.  $< 75$  percentile) was 4.23 (95% CI, 3.35–5.34), and that for the beta-cell dysfunction (HOMA-%B  $\leq 25$  percentile vs.  $> 25$  percentile) was 5.31 (4.21–6.71) after adjustment for age, gender, BMI, alcohol consumption, smoking status, physical activity, and family history of diabetes.

**Conclusion:** These results suggested that the primary pathogenetic factor of type 2 diabetes in Korean is heterogeneous with varied contribution of insulin resistance and beta-cell dysfunction according to BMI. In subjects with lower BMI, beta-cell dysfunction is the predominant defect, while insulin resistance is the predominant pathogenetic factor in subjects with higher BMI in the development of type 2 diabetes.

## 356

**Evidence for different subtypes of type 2 diabetes: prospective analysis of the Whitehall II study**K. Færch<sup>1</sup>, D.R. Witte<sup>1,2</sup>, A.G. Tabák<sup>2,3</sup>, E.J. Brunner<sup>2</sup>, M. Kivimäki<sup>2</sup>, D. Vistisen<sup>1</sup><sup>1</sup>Steno Diabetes Center, Gentofte, Denmark, <sup>2</sup>Department of Epidemiology and Public Health, University College London, UK, <sup>3</sup>Department of Medicine, Semmelweis University, Budapest, Hungary.

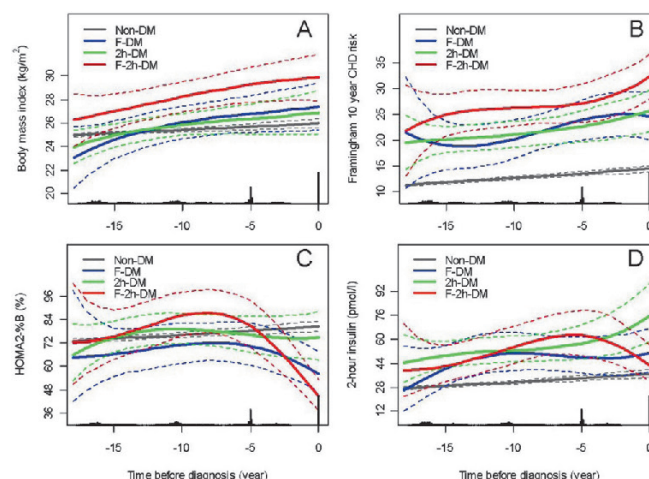
**Background and aims:** Underlying individual differences in physiology may occur early in the pathogenesis of type 2 diabetes. We determined whether different subgroups of type 2 diabetes based on the type of diagnostic glucose value exhibit different trajectories of metabolic features before the time of diagnosis.

**Methods:** Data from the prospective Whitehall II cohort were used. The study included 274 individuals who developed screen-detected type 2 diabetes over 18 years. These patients were divided into subgroups based on

their fasting and 2-hour glucose concentration at the time of diagnosis as: 1) elevated fasting glucose only (F-DM,  $n=55$ ); 2) elevated 2-hour glucose only (2h-DM,  $n=148$ ); and 3) both elevated fasting and 2-hour glucose (F-2h-DM,  $n=71$ ). Individuals without diabetes during the study were included as reference group ( $n=6,569$ ). Trajectories of measures of glucose metabolism, body mass index (BMI), and estimated risk of coronary heart disease (CHD, Framingham score) up to the time of diagnosis were studied using growth curve mixed-effects models with cubic term for time and adjusting for age, sex, ethnicity and study phase.

**Results:** The shapes of the trajectories for BMI and CHD risk were similar for all three diabetes groups, however with higher levels of BMI ( $P < 0.05$ ; Fig. 1A) and CHD risk ( $P \leq 0.002$ , Fig. 1B) in patients diagnosed by F-2h-DM. Interestingly, patients diagnosed by F-DM had a stable but low beta cell function more than a decade before their clinical diagnosis, whereas those with F-2h-DM exhibited an increased beta cell function 7–8 years before diagnosis, followed by a loss of beta cell function up to the date of diagnosis ( $P < 0.001$ ; Fig. 1C). Individuals who developed 2h-DM had slightly increasing 2-hour insulin concentration up to the time of diagnosis, whereas those with F-2h-DM experienced a fall in 2-hour insulin concentration a few years prior to their diagnosis ( $P < 0.001$ ; Fig. 1D).

**Conclusion:** Patients with type 2 diabetes show different trajectories of metabolic parameters before diagnosis based on whether they are diagnosed by elevated fasting glucose, 2-hour glucose or both, suggesting that their underlying pathophysiology may be different. These findings question whether a “one size fits all” prevention strategy can be equally effective for all people with a high risk for diabetes.



**Figure 1** Trajectories of BMI (A), 10-year risk of coronary heart disease (B), homeostasis model assessment of beta cell function (C), and 2-hour postload insulin (D) in individuals who develop different subtypes of diabetes as well as of individuals who do not develop diabetes during the study period of 18 years. Models are adjusted for age, sex, ethnicity and study phase.

Clinical Trial Registration Number: NCT00005680

## 357

**Sex difference in trajectories of glycaemic indices prior to diabetes diagnosis: the Whitehall II study**D. Vistisen<sup>1</sup>, D.R. Witte<sup>1,2</sup>, A.G. Tabák<sup>2,3</sup>, E.J. Brunner<sup>2</sup>, M. Kivimäki<sup>2</sup>, K. Færch<sup>1</sup><sup>1</sup>Steno Diabetes Center A/S, Gentofte, Denmark, <sup>2</sup>Department of Epidemiology and Public Health, University College London, UK, <sup>3</sup>Department of Medicine, Semmelweis University, Budapest, Hungary.

**Background and aims:** Isolated impaired fasting glycaemia is more common in men than in women, while the opposite is true for isolated impaired glucose regulation. Moreover, women are more commonly diagnosed with type 2 diabetes on the basis of 2 h postload plasma glucose levels compared with fasting plasma glucose levels. We sought to examine sex differences in trajectories of indices of glycaemia in individuals developing type 2 diabetes.

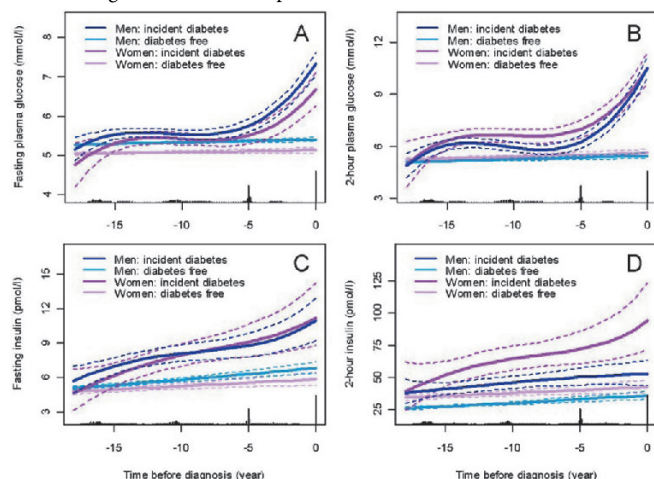
**Materials and methods:** In the prospective Whitehall II cohort of 7,364 participants, 547 men and 247 women developed diabetes during an 18-year follow-up (1991–2009). We analysed repeat data on their plasma glucose and serum insulin levels from oral glucose tolerance tests administered every 5 years, using participants who did not develop diabetes as controls ( $n=6,567$ ). Trajectories of glucose and insulin concentrations up to the date of diagnosis for incident diabetes cases and to the last date of screening for controls were



studied using growth curve mixed-effects models, with cubic term for time and adjusting for age, ethnicity and study phase.

**Results:** For fasting glucose and insulin, the shape of the curves were similar between men and women, however with higher fasting glucose levels among men over the entire follow-up (Figure 1A,  $p < 0.01$ ). In contrast, for 2-hour glucose and insulin, trajectories differed between sexes and with higher values for women, though it reached statistical significance only for 2-hour insulin (Figure 1D,  $p = 0.01$ ).

**Conclusion:** Trajectories of glucose and insulin differ between men and women before the onset of diabetes, indicating that prevention strategies for diabetes might need to be sex-specific.



**Figure 1** Trajectories of indices of glycaemia for men and women with and without diabetes during a study period of 18 years. Models were adjusted for age, ethnicity and study phase.

Clinical Trial Registration Number: NCT00005680

## 358

### Early onset type 2 diabetes and learning disability: an important and common novel clinical syndrome

A.J. Chakera, S. Irvine, S. Hammersley, M. Sheppard, UNITED research team, A.T. Hattersley;  
Peninsula College of Medicine and Dentistry, Exeter, UK.

**Background:** People with learning disability (LD) have not been recognised as having a discrete subtype of diabetes and any increased prevalence of diabetes has previously been attributed to higher rates of obesity.

**Aim:** To determine the prevalence and characteristics of patients with young-onset diabetes and learning disability.

**Methods:** Patients with LD and diabetes were identified from a population based study of patients with diabetes diagnosed <30 years. In addition we looked at the prevalence of diabetes in 1642 subjects on primary care LD registers.

**Results:** 5/45 (11.1%) patients with Type 2 diabetes (T2D) diagnosed <30 years, have learning disability requiring community support. None had known diabetes associated syndromes. This is >5-fold higher than the national prevalence of 1–2%. Patients with T2D and LD, had a lower BMI (33.5 kg/m<sup>2</sup> v 38.3 kg/m<sup>2</sup> ( $p=0.032$ )) and an earlier age at diagnosis (15.2 v 25.5 years ( $p=0.003$ )) than T2D without LD. In patients with LD the prevalence of diabetes was 99/1642 (6.0%). This is 1.5-fold higher than national prevalence in a group that has lower life expectancy and younger age distribution.

**Discussion:** We have found a strong association between young-onset Type 2 diabetes and LD in two studies ascertained by different routes. The high prevalence suggests diabetes care is an important health requirement for people with LD. The younger age of diagnosis and lower BMI suggests these patients have more severe beta-cell dysfunction than T2D in patients without LD. The association may be attributable to a previously unrecognised genetic syndrome of both diabetes and learning disability.

## 359

### Prevalence and associations of complementary and alternative medicine natural products in adults with newly diagnosed type 2 diabetes

R.N. Patel<sup>1</sup>, M.S.B. Huda<sup>1</sup>, K.F. Hunt<sup>1</sup>, K. Winkley<sup>2</sup>, K. Ismail<sup>2</sup>, S.A. Amiel<sup>1</sup>, SOUL-D Research Group;

<sup>1</sup>Diabetes Research Group, <sup>2</sup>Institute of Psychiatry, King's College London, UK.

**Background and aims:** Use of complementary and alternative medicine (CAM) in type 2 diabetes (T2DM) is high despite advances in proven conventional therapies. Its prevalence of use in the UK is unclear and its correlates with diabetes outcomes have not been investigated. We examined the utilisation of natural products (NPs), a major subcategory of CAM, in newly diagnosed T2DM and described its associations with demographic, biological and lifestyle data in a large South London cohort.

**Materials and methods:** We used data collected in the South London Diabetes (SOUL-D) cohort study which is recruiting people with newly diagnosed T2DM for the investigation of psychosocial factors in diabetes. Baseline information collected within 6 months of diagnosis of T2DM is available on 1200 participants. Data on NP use are missing for 26 patients. We subcategorised NP use into: herbs and botanicals; vitamins, minerals and oils and other. Multiple logistic regression was used to verify independent associations of NP use in individuals with T2DM, controlling for age, gender, ethnicity, biological and lifestyle factors.

**Results:** Of 1174 patients (mean duration of T2DM  $4.8 \pm 3.8$  months), 107 (9.1%) were using NPs (herbs and botanicals 30%; vitamins, minerals and oils 45%; other 13% and not specified 12%). Number of NPs consumed per person ranged from 0–6; 29% of NP users took more than one product. There were no significant differences between the NP users and the non NP users in age (mean  $56.9 \pm 10.2$  vs.  $55.4 \pm 11.6$  years), gender (male 51.4 vs. 56.2%), ethnicity (white 49.5 vs. 51.7%, African Caribbean 38.3 vs. 34.5%, Asian/other 12.2 vs. 13.8%) or occupation (professional /manager 34.3 vs. 28.9%); birth outside England (49.5 vs. 54.9%); religious identity (77.6 vs. 73.4%); diagnosis by screening (64.4 vs. 59.5%) or attendance of structured group education (12.1 vs. 17.6%). Self-employment was higher in NP users vs. non-users (27 vs. 16.8%,  $p=0.01$ ). Being a non-smoker was also significantly associated with NP use (87.4 vs. 78.8%,  $p=0.04$ ). Univariate analyses revealed better glycaemic control at diagnosis in NP users (median (IQR) HbA1c 6.7 (6.2–8.1) vs. 7.1 (6.5–8.7),  $p=0.021$ ) and correspondingly a higher HDL (1.2 (1.0–1.6) vs. 1.1 (0.96–1.4),  $p=0.016$ ). Multiple logistic regression analysis (OR [95% confidence interval],  $p$  value) found self-employment (2.14 [1.09–4.21],  $p=0.027$ ) and being a non-smoker (0.28 [0.06–0.80],  $p=0.017$ ) to be independently associated with NP use.

**Conclusion:** This is the first large prevalence study of NP users with recent onset T2DM described in a UK population. Our data show that approximately 10% of people with newly diagnosed T2DM are using NPs, significantly less than that described in other Western countries. NP use is independently associated with being self-employed and a non-smoker and may reflect a more proactive sub-group of patients with healthier behaviours.

## 360

### Optimal statin dosages and compliance: a five year retrospective cohort analysis in South Indian type 2 diabetes subjects

G. Krishnan, P. Sadasivan Pillai, A. Shankar, J. Kesavadev, S. Jothydev;  
Jothydev's Diabetes Research Center, Trivandrum, India.

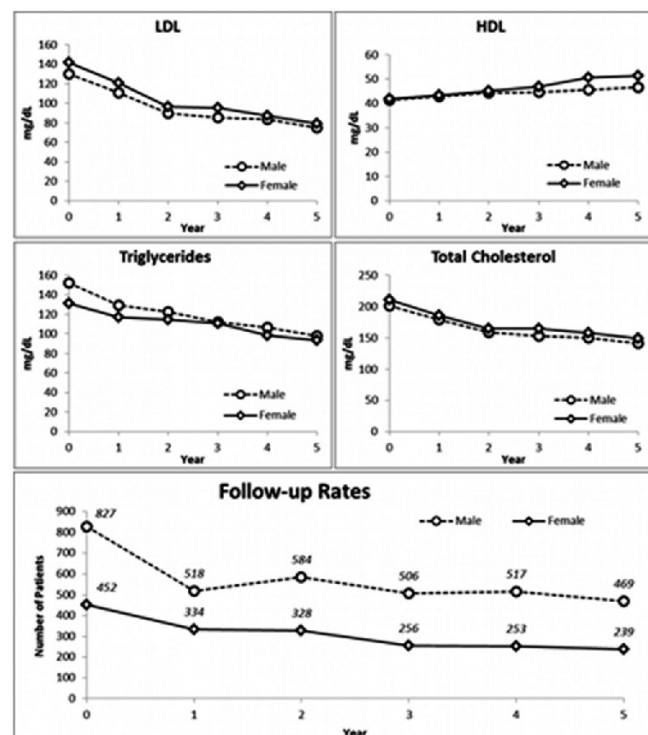
**Background and aims:** This retrospective cohort study of patients presenting in our clinic in years 2006 and 2007 was carried out to examine trends of lipid parameters, dosage requirements and compliance patterns. In our center T2DM patients with high risk are prescribed statins with a treat to target LDL goal below 70 mg/dl. Compared to other ethnicities the average statin dose requirement in Asians required to reach LDL goals may be less making it a cost effective option. One major reason for the alarming rise in cardiovascular disease epidemic in India could be attributed to irregularities in statin usage. We have earlier reported non-compliance to statin therapy in more than 60% of defaulters which is subsequent to fear of adverse effects or due to the misconception of being unnecessarily on an extra drug.

**Material and methods:** We examined de-identified records of 1279 T2DM patients above 25 years of age who were treated for dyslipidemia (65% males, mean age  $51.9 \pm 11.1$  years; 35% female, mean age  $52.6 \pm 10.8$  years). Patients were followed up via a Diabetes Tele Management System (DTMS<sup>®</sup>) by a multi-disciplinary team of doctors, dieticians, pharmacists, psychologist etc.

where in addition to the glycemic management, compliance to statins and anti-hypertensives are ensured. Follow-up rates showed sharp dropout after one or two visits to the clinic (37% males, 26% females), followed by relatively steady pattern in subsequent years. Lipid parameters of both dropouts and followed-up patients were similar initially, with average LDL levels of 135mg/dL in females and 124mg/dL in males (Fig.1).

**Results:** All patients with regular follow-up showed significant improvement in all lipid parameters, with those with 5-year follow-up showing most changes. The top three drug/dose combos and associated LDL patterns were : atorvastatin 10mg (30%; mean LDL  $74 \pm 30$  mg/dL), atorvastatin 5mg (13% subjects;  $66 \pm 22$ mg/dL) and rosuvastatin 5mg (10%;  $61 \pm 28$ mg/dL). The mean dose of atorvastatin as monotherapy among patients with LDL <100mg/dL was 14.7mg and that of rosuvastatin monotherapy was 7.2mg.

**Conclusion:** The study shows that average dosage requirements of statins in South Indian populations titrated to target lipid parameters are low, and the low compliance rate that seems to exist, pose a significant public health concern. Considering the low optimal dose of statins required in the majority of T2DM subjects to keep LDL levels at goals, this is undoubtedly a cost effective option. Widespread public health campaigns have to be promoted to maintain statin compliance among diabetes patients.



HbA1c distribution. This is illustrated in Figure 1. The median yearly HbA1c was 7.8% [95% CI: 7.7 to 7.8%]. The median HbA1c for each month was 7.8% in January, March, May and September to December and 7.7% for the rest of the months. There was no significant difference in the median HbA1c for each month ( $p=0.380$ ).

**Conclusion:** We found no variation in HbA1c across the months over the period of study. The results of the present study reaffirms that seasonal variation in HbA1c does not exist in patients with diabetes who live in tropical countries such as Singapore.

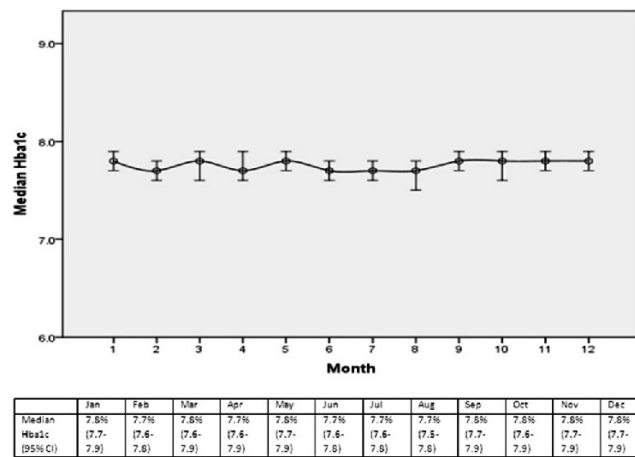


Figure 1: Monthly median HbA1c with 95% confidence intervals from 2004 to 2010

## 361

Is there seasonal variation of HbA<sub>1c</sub> in a tropical climate location such as Singapore?

D. Tay, B. Ng, L. Cho;

Changi General Hospital, Singapore.

**Background and aims:** There have been several studies examining the seasonal variability of the HbA<sub>1c</sub>. Almost all studies were done in temperate regions in both northern and southern hemispheres which showed that HbA<sub>1c</sub> varies in a sinusoidal pattern with a peak in winter and a nadir in summer. However little is known about the variations and fluctuations in HbA<sub>1c</sub> values in tropical climates such as that in Singapore in South East Asia.

**Materials and methods:** This is a retrospective study of 13886 HbA<sub>1c</sub> tests from 6646 patients who attended our diabetes service between January 2004 to March 2010. Monthly temperatures were obtained from Changi Meteorological Station. Data was analysed using SPSS version 19. One-way ANOVA test was used to compare the medians.

**Results:** There were no significant differences in monthly temperatures over the period of the study. The mean yearly temperature in Singapore was 27.8°C. Similarly there did not appear to be a seasonal variation seen in the

## PS 012 Diagnosis of type 2 diabetes

### 362

#### Casual blood glucose to identify diabetes as identified by HbA<sub>1c</sub>

T. Nakagami<sup>1</sup>, Y. Uchigata<sup>1</sup>, N. Yoshiike<sup>2</sup>;

<sup>1</sup>Diabetes Center, Tokyo, <sup>2</sup>Department of Health Science, Aomori, Japan.

**Background and aims:** Recently, casual blood glucose (CBG) over 200 mg/dL has been included in the 10-year risk assessment chart for death from cardiovascular disease (CVD) in Japan. CBG is measured routinely and thus could be used to identify individuals needing a diagnostic test for diabetes, as many clinicians only regard CBG  $\geq 200$  mg/dL as informative. The aim of this study was to assess whether lower values of CBG could be used for screening of diabetes in the integrated CVD screening program.

**Materials and methods:** 15,369 individuals (5,979 men) aged 20 years and older who had not been previously diagnosed with diabetes had measurements of both HbA<sub>1c</sub> and plasma CBG with information on postprandial time (0.5, 1, 2, 3, 4, 5–6, 7–8,  $>8$  hours), in the Japan National Health and Nutrition Survey conducted in 1997, 2002, and 2003. Individuals were categorized into four HbA<sub>1c</sub> groups as follows:  $<5.6$ , 5.6–5.9, 6.0–6.4 (=possible diabetes),  $\geq 6.5\%$  (=undiagnosed diabetes). The area under the receiver operating characteristic curve (AUC) for CBG corresponding to HbA<sub>1c</sub>  $\geq 6.5\%$  and  $\geq 6.0\%$  was calculated in three postprandial times:  $\leq 2$ , 3–4,  $\geq 5$  hours.

**Results:** The means (SDs) for age, CBG and HbA<sub>1c</sub> were 53 (16) years, 104 (29) mg/dL and 5.6 (0.7) %, respectively. The prevalence of HbA<sub>1c</sub>  $\geq 6.5$  and  $\geq 6.0\%$  and CBG  $\geq 200$  and  $\geq 100$  mg/dL were 5.4, 16.1, 1.4, and 46.2%, respectively. The mean CBGs increased with deteriorated with HbA<sub>1c</sub> categories in any postprandial time and decreased according to postprandial time until  $<5$  hours in any HbA<sub>1c</sub> category. The AUCs for CBG corresponding to HbA<sub>1c</sub>  $\geq 6.5\%$  in postprandial time  $\leq 2$ , 3–4,  $\geq 5$  hours were 87.2 (95% CI: 84.7–90.1), 86.1 (83.4–88.7) and 81.6 (78.6–84.6). The respective values corresponding to HbA<sub>1c</sub>  $\geq 6.0\%$  were 73.1 (71.0–75.3), 74.8 (72.9–76.6) and 70.6 (68.5–72.6). These were higher in postprandial time  $<5$  hours than  $\geq 5$  hours ( $p<0.05$ ). This finding was not influenced by the condition of CVD risk factors: age, hypertension, hyperlipidemia and smoking status. The table shows the performance of different CBGs corresponding to HbA<sub>1c</sub>  $\geq 6.5$  or  $\geq 6.0\%$  according to postprandial time. When CBG  $\geq 200$  or  $\geq 100$  mg/dL were used for the screening of diabetes, the positive predictive value (PPV) and the specificity increased and the sensitivity decreased according to postprandial time course. The optimal cut-off of CBG maximizing sensitivity plus specificity for HbA<sub>1c</sub>  $\geq 6.5\%$  decreased with postprandial time course: 130, 122, and 108 mg/dL. The respective values for HbA<sub>1c</sub>  $\geq 6.0\%$  were: 117, 111, and 98 mg/dL. In the data analysis of postprandial time  $\geq 5$  hours, the CBGs that provided identical prevalence of HbA<sub>1c</sub>  $\geq 6.5\%$  ( $=4.7\%$ ) and  $\geq 6.0\%$  ( $=13.7\%$ ) were 124 mg/dL and 109 mg/dL, respectively.

**Conclusion:** CBG is a useful screening test for diabetes defined by HbA<sub>1c</sub>. The lowered cut-off of CBG (around 110–130 mg/dL) could be efficiently used for the screening of diabetes in the integrated CVD screening program.

Performance of different CBGs corresponding to HbA<sub>1c</sub> over 6.5% or 6.0% by postprandial time

casual blood glucose (mg/dL)	post-prandial time (hours)	HbA <sub>1c</sub> over 6.5% (undiagnosed DM)				HbA <sub>1c</sub> over 6.0% (possible DM)			
		sensitivity (%)	specificity (%)	PPV (%)	People requiring diagnostic test (%)	sensitivity (%)	specificity (%)	PPV (%)	People requiring diagnostic test (%)
200	$\leq 2$	35	99	73	3	13	99	83	3
	3–4	24	100	100	2	8	100	99	2
	$\geq 5$	14	100	100	1	5	100	100	1
100	$\leq 2$	95	40	9	62	84	43	24	62
	3–4	90	51	11	52	79	54	27	52
	$\geq 5$	77	70	11	32	58	72	25	32
optimal cut-off point	$\leq 2$	76	85	23	19	60	74	33	32
	3–4	73	89	29	15	59	79	38	28
	$\geq 5$	64	88	21	15	65	65	23	39

Supported by: Ministry of Health, Labor and Welfare, Japan

### 363

#### A cross-sectional study for assessment and validation of the estimated formula of 2-hour post-challenge glucose level composing fasting glucose level and HbA<sub>1c</sub>

Y. Kondo<sup>1</sup>, N. Harada<sup>1</sup>, A. Hamasaki<sup>1</sup>, D. Nastesuka<sup>1</sup>, S. Yamane<sup>1</sup>, A. Muraoka<sup>1</sup>, T. Sozu<sup>2</sup>, N. Inagaki<sup>1</sup>;

<sup>1</sup>Kyoto University Graduate School of Medicine, <sup>2</sup>Biostatistics, Kyoto University School of Public Health, Japan.

**Background and aims:** Postprandial hyperglycemia begins prior to Type 2 diabetes mellitus (T2DM). Since, it is a high risk factor not only for development of T2DM but also for development of macrovascular disease, its detection and early intervention are critical for preventing pre-diabetes and reducing diabetic complications. Oral glucose tolerance test (OGTT) is an appropriate method for estimating postprandial hyperglycemia. So far, combination of fasting plasma glucose levels (FPG) and HbA<sub>1c</sub> (without OGTT) was used for DM or impaired glucose tolerance (IGT) screening. However, the calculation of estimated formula of 2-hour post-challenge plasma glucose level (2hPG) during OGTT and validation of the accuracy of the formula were not assessed. So we designed a hospital-based cross-sectional study to assess and validate the estimated formula of 2hPG composing FPG and HbA<sub>1c</sub>, and then verified whether the subjects should undergo OGTT or not.

**Materials and methods:** 380 Japanese participants undergoing 75g OGTT were recruited. Gender, age, body mass index (BMI) and HbA<sub>1c</sub> were checked. OGTT was performed and PG and serum insulin (IRI) levels were measured at 0, 30, 60, 90, and 120 min after oral administration of 75g glucose. We separated subjects into two groups randomly: Test group (n=190) for stepwise multiple linear regression analysis, and validation group (n=190) for validation between measured 2hPG (m2hPG) obtained from OGTT and estimated 2hPG (e2hPG) calculated from estimated formula. We also analyzed the sensitivity (Sn), specificity (Sp), and the receiver-operating characteristic (ROC) curve in validation group to assess the optimal cut-off values for detecting DM and IGT. Data were analyzed using JMP Statistical Software (ver. 9).

**Results:** According to the WHO criteria, the subjects were classified into the four groups: normal glucose tolerance (NGT) 32.9%, impaired fasting glucose (IFG) 2.4%, IGT 28.1%, DM 36.6%. Mean age was 54.5 $\pm$ 15.6 (mean $\pm$ SD) years and mean BMI was 23.4 $\pm$ 4.8 kg/m<sup>2</sup>. High linear correlations between m2hPG and FPG and between m2hPG and HbA<sub>1c</sub> were demonstrated, with  $r=0.75$  and  $0.70$ , respectively, which were regarded as strongest predictors of m2hPG. Multiple linear regression analysis was performed in the test group;  $e2hPG = 1.67 \times FPG \text{ (mmol/L)} + 1.63 \times HbA_{1c} - 10.1$  (adjusted  $R^2 = 0.60$ ,  $P<0.0001$ ). In the validation group, when the cut-off value was based on the diagnosis of DM (11.1 mmol/L) and IGT (7.8 mmol/L), the equation had 80% Sn and 95% Sp for detecting DM, and 86% Sn and 39% Sp for detecting IGT, respectively. ROC curve showed the optimal cut-off values of e2hPG at 10.8 mmol/L with 85% Sn and 94% Sp for detecting DM, and at 7.5 mmol/L with 92% Sn and 38% Sp for detecting IGT.

**Conclusion:** For detecting non-IGT and DM, high Sn and Sp are needed for cut-off value, respectively. The present study demonstrated that our formula is useful to judge the necessity of OGTT, that is, the values of e2hPG  $< 7.8$  mmol/L or  $11.1$  mmol/L  $< e2hPG$  could be regarded as non-IGT and DM, respectively.

Clinical Trial Registration Number: E1135 at Kyoto University

### 364

#### Fasting plasma glucose (FPG), HbA<sub>1c</sub> and OGTT in screening for type 2 diabetes: the diabetes mellitus and vascular health initiative (DMVhi) study

M. Sinnott<sup>1</sup>, B. Kinsley<sup>2</sup>, T. O'Grady<sup>1</sup>, C. Walsh<sup>3</sup>, P. Gaffney<sup>4</sup>, G. Boran<sup>4</sup>, J.J. Nolan<sup>5</sup>, B. Carr<sup>6</sup>;

<sup>1</sup>Wellness, Vhi Healthcare, Dublin, <sup>2</sup>Diabetes, Mater Misericordiae University Hospital, Dublin, <sup>3</sup>Statistics, Trinity College, Dublin, <sup>4</sup>Clinical Chemistry Laboratory, Tallaght Hospital, Dublin, Ireland, <sup>5</sup>Chief Executive, Steno Diabetes Center, Copenhagen, Denmark, <sup>6</sup>Medical, Vhi Healthcare, Dublin, Ireland.

**Background and aims:** Early diagnosis and treatment improves outcomes in T2DM. In pre-diabetes (preDM) lifestyle modification prevents or delays progression to T2DM. The DMVhi Study was undertaken by Ireland's largest health insurer to assess prevalence of T2DM and preDM and to investigate efficient approaches to population screening. The aim of this abstract is to



compare a combination of FPG, HbA<sub>1c</sub> and OGTT with FPG and OGTT as a screening method for T2DM and preDM.

**Materials and methods:** Policy holders (45–75 years, non DM) in two major urban areas were invited for screening. To date over 24,000 individuals have been screened. This abstract describes data for a sub group of 2,460 individuals where each participant had an HbA<sub>1c</sub> and FPG measured, plus OGTT if FPG  $\geq 5.6 \leq 6.9$  mmol/L or repeat FPG if  $\geq 7.0$  mmol/L.

**Results:** FPG and OGTT screening gave a DM rate = 1.3%, preDM = 5.8%, normal = 92.9%. OGTT rate = 9.8%. FPG and HbA<sub>1c</sub> screening gave a DM rate = 1.3%, preDM = 36%, normal = 62.7%. Adding OGTT to HbA<sub>1c</sub> and FPG screening in the preDM group with both IFG and HbA<sub>1c</sub> of 5.7–6.4% identified 10 extra cases of T2DM. This screening method results in a DM rate of 1.8%, preDM = 35.5%, normal = 62.7% with an OGTT rate of 6.2%.

**Conclusion:** This data may suggest that screening using FPG and HbA<sub>1c</sub> with OGTT performed in those with both FPG and HbA<sub>1c</sub> in the preDM range, increases T2DM detection rate by 38% compared to screening with either FPG and OGTT or FPG and HbA<sub>1c</sub>. Using this method the OGTT rate decreases by 37% as compared to screening with FPG and OGTT.

## 365

### Screening for diabetes and pre-diabetes with combined use of FPG and quantitative postprandial urine glucose in Chinese high-risk population: a community-based study

J. He<sup>1</sup>, Z. Sun<sup>1</sup>, T. Wu<sup>2</sup>, Z. Xie<sup>1</sup>, Y. Lu<sup>3</sup>, C. Lei<sup>1</sup>, J. Han<sup>1</sup>, Y. Zhou<sup>1</sup>;

<sup>1</sup>Endocrinology, Institute of Diabetes, Zhongda Hospital, Nanjing, China,

<sup>2</sup>Discipline of Medicine, University of Adelaide, Australia, <sup>3</sup>Hi-Tech Research Institute, Nanjing University of Technology, China.

**Background and aims:** Fasting plasma glucose (FPG) has not been sensitive enough for diabetes screening, while measurements of oral glucose tolerance test (OGTT) and HbA<sub>1c</sub>, although reliable, are time-consuming and resource-dependent, appearing less practical in large-scale screening, especially in low-income regions. The study was designed to evaluate the utility of the combination of FPG and quantitative postprandial urine glucose (PUG) in diabetes screening among Chinese high-risk population.

**Materials and methods:** Subjects with high risks but no previous diabetes history were recruited from 8 Chinese community medical centers from September 2010 to September 2011 for the measurements of FPG, 75 g-OGTT, HbA<sub>1c</sub> and 2h-PUG after 75 g oral glucose intake. The ROC curve was used to evaluate the performance of PUG in diabetes screening, from which an optimal threshold of PUG was estimated. Combined use of FPG and PUG in diabetes and pre-diabetes screening was evaluated against FPG alone.

**Results:** 909 out of 1035 subjects completed the study. The levels of 2h-PUG in response to 75g oral glucose positively correlated to FPG, 2h-PG, and HbA<sub>1c</sub>, respectively ( $P < 0.001$  for each). A threshold of 2h-PUG at 135 mg/dL was estimated to detect a 2h-PG  $\geq 11.1$  mmol/L, with a sensitivity of 76.2% and a specificity of 89.4%. In contrast to FPG, combined use of FPG ( $\geq 5.6$  mmol/L),  $< 7.0$  mmol/L, and PUG ( $\geq 135$  mg/dL) had a comparable capacity to detect glycemic dysregulation, with a sensitivity of 84.9% and specificity of 66.3% to identify a 2h-PG  $\geq 11.1$  mmol/L, and a sensitivity of 68.6% to predict postprandial hyperglycemia (2h-PG  $\geq 7.8$  mmol/L). Both sensitivity was higher than applying FPG test alone in screening ( $P < 0.0000$  for each comparison).

**Conclusion:** The combined use of FPG and PUG shows a good performance in diabetes and pre-diabetes screening, although its specificity for diabetes diagnosis is relatively low. The measurements of FPG and PUG may not replace the diagnostic value of OGTT, but represents a promising method for diabetes and pre-diabetes screening among high-risk population.

Clinical Trial Registration Number: BK 2010087

Supported by: The Key Program of Jiangsu Natural Science Foundation

## 366

### Post glucose-load hypoglycaemia is not associated with increased incidence of diabetes after three years. The DEPLAN study

S. Liatis, C. Stathi, A. Tsiakou, D. Perrea, N. Katsilambros, K. Makrilakis; First Department of Internal Medicine, Diabetes Center, Athens, University Medical School, Laiko Hospital, Greece.

**Background and aims:** It has been reported that postprandial (reactive) hypoglycaemia may be related to future development of diabetes. Aim of the present analysis was to investigate the relationship between hypoglycaemia

that develops two hours after an oral glucose tolerance test (OGTT) and incidence of diabetes or impaired glucose tolerance (IGT) after 3 years.

**Materials and methods:** The study participants were healthy individuals who underwent an OGTT during a screening procedure for participation in the Greek arm of a European, community-based, type 2 diabetes prevention programme by lifestyle modification (DEPLAN). The OGTT was repeated 3 years later. Post-load hypoglycaemia was defined as a plasma glucose value  $\leq 70$  mg/dl at two hours. IGT was defined as a plasma glucose value 140–199 mg/dl at two hours post-load.

**Results:** The analysis was performed in 229 individuals (116 males, mean age: 53.67 [52.37–54.98] years, mean BMI: 29.43 [28.76–30.10] Kg/m<sup>2</sup>), with normal baseline glucose tolerance (NGT). At baseline, 37 individuals (16.16%) had 2hr post-load hypoglycaemia. Three-year incidence of diabetes and/or IGT was lower in those with 2hr post-load hypoglycaemic plasma glucose levels at baseline (3/37 [8.11%] vs. 43/189 [22.75%], OR: 0.309 [0.090–1.056],  $p=0.06$ ). After adjustment for age, gender, family history of diabetes, BMI, and fasting plasma glucose at baseline, 2hr post-load hypoglycaemia was not associated with increased risk for diabetes and/or IGT development after three years (OR: 0.49 [0.14–1.80]).

**Conclusion:** Individuals who exhibit hypoglycaemia two hours after a 75g oral glucose load do not seem to be at increased risk for diabetes development in the short-term (up to three years). Analyses in larger cohorts for longer periods of time are needed, in order to expand the investigation in sub-populations and draw more solid conclusions.

Supported by: EU

## 367

### Utility of HbA<sub>1c</sub> as a screening tool for diagnosis of diabetes and prediabetes in Indian population

R. Rajput;

PGIMS, Rohtak, India.

**Background and aims:** The aim of study was to evaluate the performance of HbA<sub>1c</sub> and FPG tests as a mass screening tool for diabetes and pre-diabetes as defined by standard oral glucose tolerance test (OGTT).

**Materials and methods:** Data from 1008 participants of age 20–75 years enrolled in population based cross sectional study from urban areas of Rohtak city, Haryana were analysed. 2-hr.75 gm OGTT was used to diagnose diabetes and pre-diabetes. Performance of HbA<sub>1c</sub> and FPG was evaluated against the results of OGTT by using receiver operating characteristic curve (ROC) analysis.

**Results:** The prevalence of pre-diabetes and newly diagnosed diabetes was found 20.6% and 12.5% respectively. For subjects with newly diagnosed diabetes, the area under the ROC curve was 0.957 for A1C and 0.942 for FCG ( $P$  value 0.11), whereas for pre-diabetes, these values were 0.831 for HbA<sub>1c</sub> and 0.807 for FPG ( $p$  value 0.205). At the optimal HbA<sub>1c</sub> cutoff points of 5.4% for pre-diabetes and of 6.2% for newly diagnosed diabetes, sensitivities (specificities) were 79.02%(79.31%) and 92.24%(90.52%) respectively. Similarly FPG optimal cut off points of  $\geq 97$  mg% for pre-diabetes and  $\geq 119$  mg% for diabetes were found to have max. sensitivities (specificities) of 93.17(63.32) and 93.54(89.64) respectively.

**Conclusion:** As a screening tool for newly diagnosed diabetes and pre-diabetes, the HbA<sub>1c</sub> measurement performed not inferior than FPG in this study population.

## 368

### Glycated HbA<sub>1c</sub> for diagnosing diabetes in 1329 Chinese subjects over 50 years old: a community-based cross-sectional study

S. Lin, X. Li, L. Hu, S. He, Z. Ren, X. Tang, Y. Qiu, J. Xu, P. Mu, L. Zeng; Department of Endocrinology, The 3rd Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

**Background and aims:** Recently, substantial evidence shows that glycated haemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) may be a useful tool for screening for and diagnosis of diabetes. However, little is known about using HbA<sub>1c</sub> to screen for diabetes and/or impaired fasting glucose (IFG) in Chinese subjects over 50 years old. This study aims to evaluate HbA<sub>1c</sub> in diagnosing diabetes and identify the optimal HbA<sub>1c</sub> cut-off to be used in Chinese subjects aged over 50 years in a community-based setting.

**Materials and methods:** A community-based cross-sectional epidemiological survey was conducted during Oct 2010 to Jan 2011 in Shipai community

of Guangzhou city, Guangdong province, South China. A total of 1494 subjects aged over 50 years were investigated. All participants completed a uniform questionnaire containing questions about the histories of current and previous illness and medical treatment. Fasting blood samples were obtained to measure plasma glucose and HbA<sub>1c</sub>. Plasma glucose was measured by glucose oxidase method and HbA<sub>1c</sub> was measured by using high performance liquid chromatography (D-10, BIO-RAD, America, reference range was 4.0–6.0%). The glycaemic thresholds for diagnosis of diabetes and IFG were based on the 1999 World Health Organization criteria. Diabetes is defined as fasting plasma glucose (FPG) of at least 7.0 mmol/L. 161 subjects (10.8%) had previously diagnosed with diabetes and 4 with missing data were excluded and data from 1329 subjects (50–96 years old) were analysed. Pearson correlation analysis was done to investigate the association of HbA<sub>1c</sub> with FPG; Receiver Operating Characteristics curve (ROC) were plotted to assess the sensitivity and specificity of HbA<sub>1c</sub> in identifying participants as having undiagnosed diabetes or IFG.

**Results:** 1329 subjects had average age 62 years (interquartile range: 55–67 years), mean FPG 5.2 mmol/L (SD 1.3 mmol/L), mean HbA<sub>1c</sub> 5.8% (SD 0.75%), and were 60% female. Among them, 54 subjects (4.1%) had diabetes, 88 subjects (6.6%) had IFG, 10.7% had hyperglycemia (diabetes plus IFG). HbA<sub>1c</sub> was significantly positively correlated with FPG (correlation coefficient was 0.784,  $P < 0.001$ ). According to the receiver operating characteristics curve, the area under the curve for HbA<sub>1c</sub> in diagnosing diabetes and hyperglycemia (diabetes plus IFG) were 0.945 (95% CI 0.910–0.981) and 0.849 (95% CI 0.810–0.889) respectively. An HbA<sub>1c</sub> threshold of 6.5% yielded the highest combination of sensitivity (83.3%) and specificity (95.8%) for diagnosing diabetes (with a highest Youden's index of 0.791). At an optimal cut-off point of  $\geq 6.1\%$ , the sensitivity was 71.8% and specificity 83.1% for diagnosing hyperglycemia (with a highest Youden's index of 0.549).

**Conclusion:** An HbA<sub>1c</sub> threshold of 6.5% was highly specific and have a good sensitivity for diagnosing diabetes in Chinese adults aged over 50 years in community-based setting. This optimal HbA<sub>1c</sub> threshold may be suitable as a diagnostic criterion for diabetes in elderly Chinese people when fasting plasma glucose and oral glucose tolerance tests are not available.

## PS 013 Risk scores for type 2 diabetes and disease complications

369

### Comparison of the Leicester risk assessment score, FINDRISC and SCOUT DS\* for diabetes screening in an at-risk Greek population

P. Lathouris<sup>1</sup>, K. Tzemos<sup>2</sup>, J.D. Maynard<sup>3</sup>, N. Tentolouris<sup>4</sup>;

<sup>1</sup>Central Diabetes Center, Greece National Social Insurance Institute, Athens, Greece, <sup>2</sup>KTIC, Athens, Greece, <sup>3</sup>R&D, VeraLight, Inc., Albuquerque, USA,

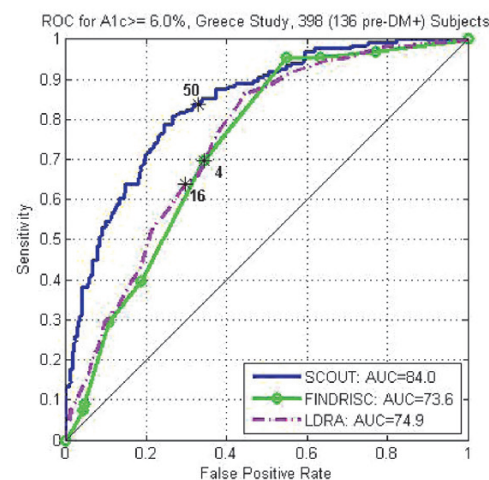
<sup>4</sup>Internal Medicine, Laiko General Hospital, Athens, Greece.

**Background and aims:** An important part of diabetes prevention is screening of at risk subjects to detect disease development at an early stage. Methods that are noninvasive, non-fasting, provide immediate results and simple to administer can facilitate ascertainment of at risk individuals. We examined the accuracy of Leicester Diabetes Risk Assessment score (LDRA), the concise Finnish Diabetes Risk Score (FINDRISC) and the skin fluorescence based SCOUT DS diabetes score for detecting increasing levels of dysglycemia as defined by HbA<sub>1c</sub>.

**Materials and methods:** Subjects at risk for type 2 diabetes but without an existing diagnosis of diabetes were recruited at the diabetes centers for the LAIKO General Hospital and Greece National Social Insurance Institute in Athens, Greece. ~200 subjects were recruited at each clinical site. Subject participation consisted of a single, non-fasting visit that lasted less than 30 minutes. The clinic staff measured the subject's height, weight and waist circumference. Skin fluorescence was measured on the underside of the left forearm with the SCOUT DS device and it calculated a diabetes score. A finger prick was done to measure RCG (OneTouch Ultra) and HbA<sub>1c</sub> (DCA Vantage). A health history questionnaire was completed by each subject to characterize cohort demographics. We used concise FINDRISC score (does not require physical activity and consumption of fruits, vegetables and berries data) because we did not collect information on consumption of fruits, vegetables and berries. A point of care HbA<sub>1c</sub> measurement was used as the reference and dysglycemia states of  $\geq 6.0\%$  and  $\geq 6.5\%$  were defined.

**Results:** 398 of 409 subjects had complete data for analysis with means and 95% confidence intervals for A1c, age, BMI, and waist of  $5.7 \pm 1.2\%$ ,  $52.3 \pm 29.4$  yrs,  $27.3 \pm 9.8$  kg/m<sup>2</sup> and  $90 \pm 26.8$  cm. 51% were male and the cohort was 99.5% Caucasian. Prevalence of HbA<sub>1c</sub>  $\geq 6.0\%$  and  $\geq 6.5\%$  were 34% and 12%, respectively. The receiver operator characteristic curves for detection of A1c  $\geq 6.0\%$  are shown in Figure 1. SCOUT DS had significantly greater area under the curve (AUC) for both levels of dysglycemia relative to the LDRA and FINDRISC. For detection of HbA<sub>1c</sub>  $\geq 6.0\%$ , the SCOUT DS, LDRA and FINDRISC AUCs were 84.0%, 74.9% and 73.6%, respectively. For detection of HbA<sub>1c</sub>  $\geq 6.5\%$ , the SCOUT DS, LDRA and FINDRISC AUCs were 90.4%, 78.6% and 79.4%, respectively (ROCs not shown).

**Conclusion:** All methods were able to detect HbA<sub>1c</sub>-defined dysglycemia and have the benefits of being simple, noninvasive, non-fasting and providing immediate results at the point of service. At the respective screening thresholds, SCOUT DS detected 20% and 30% more cases of HbA<sub>1c</sub>  $> 6.0\%$  compared to FINDRISC and LDRA with comparable specificity. In this study, SCOUT DS had superior detection capability relative to the questionnaire-based LDRA and concise FINDRISC scores.



## 370

**Risk scores for diabetes and impaired glycaemia in the Middle East and North Africa**

L.N. Handlos<sup>1</sup>, D.R. Witte<sup>1</sup>, T.P. Almdal<sup>1</sup>, L.B. Nielsen<sup>2</sup>, S.E. Badawi<sup>3</sup>, A.R.A. Sheikh<sup>4</sup>, M. Belhadj<sup>5</sup>, D. Nadi<sup>6</sup>, S. Zinai<sup>7</sup>, D. Vistisen<sup>1</sup>; <sup>1</sup>Steno Diabetes Center, Gentofte, Denmark, <sup>2</sup>Novo Nordisk, Bagsvaerd, Denmark, <sup>3</sup>UAE Ministry of Health, Dubai, United Arab Emirates, <sup>4</sup>King Abdul Aziz University Hospital, Jeddah, Saudi Arabia, <sup>5</sup>EHU, Oran, Algeria, <sup>6</sup>Algerian Ministry of Health, Algiers, Algeria, <sup>7</sup>Novo Nordisk, Algiers, Algeria.

**Background and aims:** The world's highest prevalence of diabetes is found in the Middle East and North Africa (MENA) region. We aimed to develop a simple and non-invasive risk score for diabetes and impaired glycaemia in the MENA region. Furthermore, we aimed to derive national risk scores for Algeria, Saudi Arabia (KSA) and the United Arab Emirates (UAE).

**Materials and methods:** Between November 2010 and December 2011, 7,216 individuals between 30 and 75 years of age were screened in Algeria, KSA and UAE. Screening was based on opportunity sampling and consisted of a self-administered questionnaire and a clinical examination including measurement of HbA<sub>1c</sub>. One regional and three national risk scores were developed in a stepwise approach. First univariate associations between diabetes or impaired glycaemia (HbA<sub>1c</sub> ≥ 6.0%) and each of the potential risk factors were assessed separately. Secondly, risk factors significant at a 10% level in the univariate analyses were included in a multiple logistic regression using stepwise backward elimination. A p value of <5% was considered significant. To ensure a parsimonious model, risk factors which at the same time added less than 1% to the area under the receiver operating characteristics curve (AUC) and less than 5% to the relative integrated discrimination improvement were successively removed. The risk factors in the final model were each assigned a score by multiplying the regression coefficients by 10 and rounded off to the nearest integer. Each participant was assigned a summed risk score calculated by adding the individual scores of the risk factors in the model. A cut-point value on the risk score corresponding to a sensitivity as close to 75% as possible was chosen. The AUC of the MENA and the national risk scores were compared in data from each country using DeLong's method.

**Results:** After exclusion of those with known diabetes and missing data, the analyses included 6,588 (91%) out of the 7,216 individuals who were screened. An average 54.8% were men, mean age was 44.3 years, 10.0% had impaired glycaemia (HbA<sub>1c</sub> ≥ 6.0% and < 6.5%) and 7.8% had undetected diabetes (HbA<sub>1c</sub> ≥ 6.5%). The final risk scores included different combinations of age, BMI, family history of diabetes, gender, gestational diabetes, ownership of computer, self-assessed health and ethnicity (Asian or other). AUC for the MENA score was between 0.69 (95% CI: 0.66–0.72) and 0.70 (0.67–0.73). For sensitivities around 75%, specificities varied from 52% (50%–54%) to 53% (51%–55%) according to country. Generally the MENA and the national risk scores performed equally well in the three samples. In KSA, the national risk score performed significantly better (p<5%) than the MENA risk score, but the actual difference in AUC was very small, and the discrepancy in performance mainly existed at sensitivities below 70%.

**Conclusion:** A regional score for diabetes and impaired glycaemia in the MENA region was developed. The MENA score performed as well as the nationally derived risk scores from Algeria, KSA and UAE.

*Supported by: Novo Nordisk*

## 371

**Screening questionnaires are useful in identifying undiagnosed HbA<sub>1c</sub> diagnosed diabetes in a Caucasian population**

B.A. Knight, B. Shields, G. Baker, A. Hattersley, A. Jones; Peninsula Clinical Research Facility, Peninsular Medical School, Exeter, UK.

**Background and aims:** Type 2 diabetes is a common metabolic disorder which may go undiagnosed and untreated for many years, leading to increased morbidity and mortality. Early detection and instigation of treatment may reduce these risks and the World Health Organisation (WHO) now recommends the use of an HbA<sub>1c</sub> value of 48mmol/mol (6.5%) as the cut off value for the diagnosis of diabetes. The Leicester Risk Assessment (LRA) Score and the Cambridge Risk Assessment (CRA) Score have been developed as simple non invasive screening tools which utilise routinely available clinical characteristics to identify those at risk of undiagnosed type 2 diabetes. They were developed using OGTT (rather than HbA<sub>1c</sub>) to define diabetes. We aimed to assess the ability of these two risk scores to detect diabetes de-

fined by HbA<sub>1c</sub> in a predominantly Caucasian population without known diabetes.

**Materials and methods:** Baseline clinical data for both scoring systems (including age, sex, ethnicity, family history, BMI, waist circumference, known hypertension, current medication and smoking history) was available on 2130 participants aged 40–75 years of age without known diabetes from the Exeter Ten Thousand (EXTEND) research study. We calculated the LRA and CRA scores for all participants. We determined area under receiver operating characteristic curve (ROC AUC) for detection of diabetes (defined by HbA<sub>1c</sub> ≥ 48mmol/mol (6.5%)) for each risk score. We determined sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios for each risk score in detecting diabetes using the suggested LRA score cut off for diabetes of ≥14 and the equivalent CRA score of ≥0.127.

**Results:** Baseline characteristics of participants were (mean (SD)): age 57.0 (9.4) years, BMI 26.7 (4.4), 99% Caucasian and 36% Male. Prevalence of undiagnosed HbA<sub>1c</sub> defined diabetes was 3.1%. AUC ROC for undiagnosed diabetes was 0.80 (perfect test = 1.0) for the LRA score and 0.83 for the CRA score. Using their suggested cut offs for type 2 diabetes gave 83.6% sensitivity and 58.5% specificity (LRA score), and 83.3% sensitivity and 58.1% specificity (CRA score). PPV and NPV for both scores were identical (6.1% and 99.1% respectively). Positive and negative likelihood ratios were 2.0 and 0.28 (LRA score) and 2.16 and 0.21 (CRA score).

**Conclusion:** Both the LRA and CRA scores are potentially useful screening tools with moderately high sensitivities and negative predictive values but poor specificity. A positive result from these screening tools doubles the probability that a patient has undiagnosed diabetes from 3 to 6%. A negative result reduces the probability of undiagnosed diabetes to <0.9%. Given the poor specificity and availability of an inexpensive diagnostic test (HbA<sub>1c</sub>) selective testing other than on the basis of age may not be justified. Further assessment is needed of the utility and cost effectiveness of these scoring systems.

## 372

**Prediction of incident diabetes mellitus in nondiabetic Asian subjects: a 4-year retrospective, longitudinal study**

M. Seo, W.-S. Jeon, S. Park, E. Rhee, C. Park, K. Oh, S. Park, S. Kim, W.-Y. Lee; Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.

**Background and aims:** Prediction rules for Type 2 diabetes (T2DM) have been developed. But we lack consensus for the most effective approach in non-diabetic Asian. Our objective was to assess T2DM prediction models in non-diabetic Asian.

**Materials and methods:** A total of 5596 Koreans without diabetes who underwent consecutive comprehensive health check-ups annually for 5yr were enrolled. There were 332 cases of new T2DM, and regression models were used to predict new T2DM, starting with characteristics known to the subject (personal model, ie, age, sex, and body mass index (BMI)), adding simple clinical measurements that included metabolic syndrome traits (simple clinical model), and, finally, assessing complex clinical models that included the homeostasis model assessment-insulin resistance (HOMA-IR), fasting serum insulin level, and diagnosis of non-alcoholic fatty liver disease by ultrasonography. Discrimination was assessed with area under the receiver operating characteristic curves (AROCs).

**Results:** The personal model variables statistically significant predictors of T2DM (AROC, 0.672). In the simple clinical model, the point estimate of AROC was 0.801. Complex clinical models showed no further improvement in model discriminations (AROC 0.804) and were not superior to the simple clinical model.

**Conclusion:** We found that age, sex, obesity, and metabolic traits effectively predict T2DM risk in Asian and were used to develop a simple T2DM prediction algorithm to estimate risk of new T2DM during a 5 year follow up interval.

**Comparison of prediction of T2DM according to Clinical variables**

Model (variables)	AROC
Personal (age, sex, BMI)	0.672
Simple (personal added hypertension, low level of high-density cholesterol, elevated triglyceride levels, increased waist circumference and impaired fasting glucose)	0.801
Complex (Simple added HOMA-IR, fasting serum insulin and NAFLD)	0.804



## 373

**Characteristics and treatment of patients with type 2 diabetes and renal impairment in the UK**H.T. Smith<sup>1</sup>, S. Davé<sup>2</sup>, I. Eriksson<sup>2</sup>, S. Lawson<sup>1</sup>, A. Martin<sup>1</sup>, A. Lawton<sup>1</sup>, J. Cid-Ruzafa<sup>2</sup>, A. Maguire<sup>2</sup>;<sup>1</sup>GlaxoSmithKline, London, <sup>2</sup>United BioSource Corporation, London, UK.

**Background and aims:** Among patients with type 2 diabetes (T2DM) 30–50% have evidence of renal impairment. Options for management of hyperglycaemia in patients with T2DM and renal impairment are limited; higher levels of a drug or its active metabolites can increase the risk of adverse events and dose adjustments to improve tolerability are common in drugs cleared by renal excretion, potentially reducing efficacy. A number of commonly used drugs for T2DM are not recommended in patients with end stage renal disease. The objective of the study was to estimate the prevalence and severity of renal impairment in patients with T2DM in the UK and to describe differences between patients with and without renal impairment in terms of characteristics, treatment, comorbidities, and healthcare resource utilisation. This abstract describes the characteristics and treatment of patients with T2DM and renal impairment in the UK.

**Materials and methods:** This is a cross-sectional study. The study population comprised patients with T2DM, diagnosed or treated on or prior to the cross-sectional study date (1/7/2010). The medical history was observed for the entire record prior to/on the cross sectional date. Patients with T2DM were identified by diagnosis codes and prescription history. Stage (1 to 5) specific chronic kidney disease (CKD) Read codes were used to define renal impairment (or absence, stage 0) within the T2DM population; for patients without a Read code, eGFR was estimated and used to classify degree of renal impairment.

**Results:** 150,084 patients with T2DM were identified, a CKD stage could be identified for 127,370 (85%). Mean age was 66.9 years and 55.6% of the population were male. 47% of the T2DM patients in the study were defined as having CKD, the majority having moderate disease (stage 2–3). Patients with CKD were older than those without and age increased with CKD stage. Based on BMI 51% of patients were classed as obese ( $\geq 30$  kg/m<sup>2</sup>), the proportion was slightly higher in patients with CKD (54%). Only 54% of patients achieved target HbA1c of  $\leq 7\%$ . Prescribing varied according to CKD stage. Prescription of metformin fell with more advanced CKD and insulin prescribing was higher in the more severe CKD stages. Prevalence of several diabetes related comorbidities including coronary heart disease, congestive heart failure, peripheral vascular disease and retinopathy/blindness was higher in the CKD patient population, increasing with CKD severity.

Table 1: Patient characteristics and diabetes treatment

	No CKD						All CKD patients	All patients
	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5		
N (%)	68,089 (53)	3,069 (2)	15,803 (12)	36,304 (29)	3,332 (3)	773 (1)	59,281 (47)	127,370 (100)
Age, years; mean (SD)	63.4 (12.63)	60.8 (13.89)	66.8 (12.43)	72.7 (10.43)	77.7 (9.01)	73.6 (10.62)	70.8 (11.75)	66.9 (12.77)
% Male	54.8	53.9	60.5	55.6	48.2	59.5	56.5	55.6
BMI, kg/m <sup>2</sup> ; mean (SD)	30.6 (6.21)	29.6 (6.68)	30.9 (6.44)	31.7 (6.45)	31.9 (6.79)	31.3 (6.27)	31.4 (6.50)	30.9 (6.36)
HbA1c (% at target = 7%)	53.7	47.0	49.8	56.0	52.0	61.2	53.7	53.7
<b>Treatment at 1/7/2010 (% on therapy)</b>								
Metformin	60.7	65.7	63.3	55.1	18.2	8.4	55.1	58.1
Sulfonylureas	27.3	34.0	31.9	32.8	40.9	32.9	33.1	30.0
DPP-4 inhibitors	3.9	4.5	4.2	3.5	2.3	0.8	3.6	3.8
Other OADs	11.2	12.4	13.0	12.0	10.7	6.3	12.1	11.6
GLP agonists	1.5	2.4	2.3	1.5	0.9	0.1	1.7	1.6
Short acting insulin	1.8	2.8	2.3	2.3	5.1	6.5	2.5	2.1
Long / intermediate acting insulin	6.5	9.7	10.3	11.2	27.6	28.5	12.0	9.0
<b>Co morbidities recorded in medical history (% patients)</b>								
Renal disorders*	0.4	3.8	4.0	3.6	11.6	66.4	5.0	2.6
Coronary heart disease	17.2	17.9	24.1	30.5	41.3	39.7	28.9	22.6
Congestive heart failure	2.2	2.4	4.9	8.9	22.5	22.5	8.5	5.1
Peripheral vascular disease	10.0	13.0	14.9	18.1	26.4	29.8	17.6	13.5
Hypertension	31.3	32.5	33.9	33.5	33.3	38.9	33.6	32.4
Other cardiovascular disorders~	24.6	25.7	30.8	35.7	46.6	48.3	34.6	29.3
Retinopathy/blindness	21.1	24.7	26.7	27.5	39.3	43.9	28.0	24.3
Neuropathy	4.5	5.6	6.8	7.5	12.4	16.0	7.6	5.9

\* Acute renal failure, renal failure and other renal disorder

~ Revascularization, acute coronary syndrome, cerebrovascular disease, myocardial infarction, hypercholesterolemia, amputation

**Conclusion:** Almost half of the T2DM patient population had some degree of CKD and management of HbA1c was poor; approximately half of patients achieving HbA1c treatment goals. The CKD population exhibited higher rates of comorbidities. Limited approved treatment options are available for these patients. Despite contraindications, metformin use was predominant in the CKD population and there was an increased use of insulin compared to the broader T2DM population.

Supported by: GlaxoSmithKline

## 374

**Prevalence of chronic kidney disease in type 2 diabetes - results from a nationwide survey in Germany**L.F. Merker<sup>1</sup>, B. Gallwitz<sup>2</sup>, B. Waldeck<sup>3</sup>, K. Schoene<sup>3</sup>;<sup>1</sup>Diabetes- und Nierenzentrum Dormagen, <sup>2</sup>Medizinische Klinik 4, Eberhard Karls Universität Tübingen, <sup>3</sup>Boehringer Ingelheim Pharma GmbH & Co KG, Ingelheim, Germany.

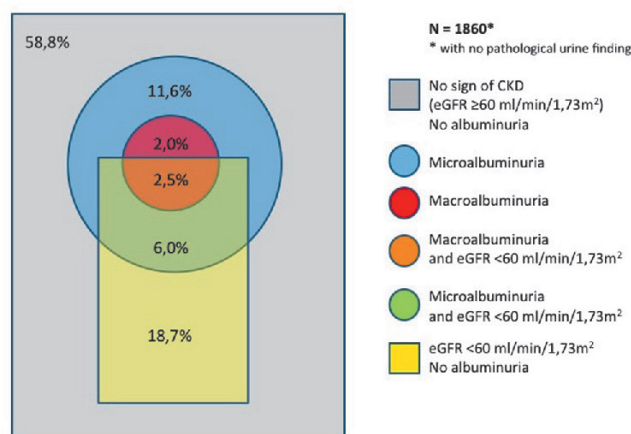
**Background and aims:** Chronic kidney disease in type 2 diabetes (T2D) is common, however, in primary care settings renal disease is frequently under-recognized and the true prevalence remains vague. In Germany a systematic prospective nationwide database on the prevalence of chronic kidney disease (CKD) in type 2 diabetes is lacking.

**Materials and methods:** To evaluate the frequency of CKD in T2D we performed a large, pre-defined and prospective nationwide survey. 2541 individuals with type 2 diabetes were recruited from 245 primary care sites. Renal screening habits prior to this survey were assessed by questionnaire. On site urinalysis was evaluated by dipstick. Central renal lab included serum creatinine, and urine albumin-creatinine-ratio (UACR); eGFR (ml/min/1.73m<sup>2</sup>) was estimated by MDRD formula. The study was conducted according to GCP standards and approved by German ethics committees. All statistical analyses were descriptive. The study was performed between February and July 2011.

**Results:** Data were available for 2531 individuals (1345 male, 1186 female). Baseline characteristics of the overall population were: Age 69.5 yrs., BMI 30.5 kg/m<sup>2</sup>, HbA1c 6.7 %, diabetes duration 8.3 years. 83 % had hypertension, 98 % received antihypertensive treatments. 1) Assessment of renal function: Prior to this study screening for renal function was conducted by serum creatinine measurement in 97%, by eGFR estimation in 51% and by Cockcroft-Gault calculation in 9%. In the study moderate renal impairment (RI) (CKD 3, eGFR 30–60 ml/min/1.73m<sup>2</sup>) was present in 27.7 %. 43.7 % of the affected had CKD diagnosis prior to this study, 55.3% were newly diagnosed. Severe RI (CKD 4, eGFR 15–30 ml/min/1.73m<sup>2</sup>) was present in 1.4 % with the magnitude previously diagnosed (91.4 % vs. 8.6% newly diagnosed). 2) Assessment of renal damage: Prior to this study screening for albuminuria was conducted in 54% by dipstick and in 7% by laboratory assessment. In the study elevated UACR (>30mg/g creatinine) was detected in 22.2 %, with micro- and macroalbuminuria being present in 17.6 % and 4.5% respectively. When considering signs of renal function decline and damage, 40.8% were affected by CKD: Micro- and macroalbuminuria associated with CKD was present in 6% and 2.5%. eGFR <60 ml/min/1.73m<sup>2</sup> without albuminuria was found in 18.7 % and albuminuria with eGFR >60 ml/min/1.73m<sup>2</sup> in 13.6%.

**Conclusion:** Based on these results, CKD is a common condition in T2D in Germany with 4 out of 10 patients being affected. However, in more than half of all patients being identified with moderate renal impairment this condition was previously not diagnosed. Assessing renal function using both eGFR and screening for albuminuria is most appropriate to detect renal disease in T2D. This should be mandatory particularly in elderly patients to detect renal impairment, which is necessary e.g. for dose adjustments of medication or referral to nephrologists.

## Distribution of renal insufficiency and albuminuria



Supported by: BI Pharma GmbH & Co KG

## 375

### Glomerular filtration rate-dependent association of serum uric acid with metabolic syndrome and hepatic fat content in Chinese community population

M.-F. Xia<sup>1</sup>, H.-D. Lin<sup>1</sup>, X.-M. Li<sup>1</sup>, H.-M. Yan<sup>1</sup>, H. Bian<sup>1</sup>, W.-Y. He<sup>2</sup>, X. Gao<sup>1</sup>;  
<sup>1</sup>Endocrinology, <sup>2</sup>Ultrasonography, Zhongshan Hospital, Shanghai, China.

**Background and aims:** Mounting evidence has demonstrated that hyperuricaemia was involved in the development of metabolic syndrome (MS), type 2 diabetes and non-alcoholic fatty liver disease (NAFLD). All these conditions are thought to be partly mediated by the pro-oxidant effect of uric acid. However, uric acid is also a special molecule with a dual role both as a pro- and as an anti-oxidant under different physicochemical circumstance, and some recent studies indicated a different contribution of hyperuricaemia for the occurrence of metabolic disorders between subjects with diet and lifestyle-induced uric acid over-production and those with uric acid retention due to impaired renal excretion function. The aim of this study was to detect the association of serum uric acid level with MS and NAFLD in a middle-aged and elderly population with normal and impaired renal excretion function, respectively.

**Materials and methods:** A cross-sectional study was performed in a community population comprising 1141 subjects enrolled consecutively to Shanghai Changfeng Study in one year. Participants with hepatitis B or C, excessive alcohol intake and other hepatic disease or use of hepatic protectant or hypouricemic agents were excluded. A standard interview, anthropometric, laboratory measurement and hepatic fat content quantification by a newly-established were performed for each participant.

**Results:** ① Serum uric acid was significantly associated with hepatic fat content ( $r=0.193$ ,  $P<0.001$ ) and higher in subjects with metabolic syndrome than those without metabolic syndrome ( $P<0.001$ ) by univariate correlation analysis and independent-sample t-test, especially in subjects with normal renal excretion function ( $\text{GFR} \geq 90 \text{ ml/min/1.73m}^2$ ) ( $r=0.255$ ,  $P<0.001$ ). ② Logistic regression analysis showed that in subjects with  $\text{GFR} \geq 90 \text{ ml/min/1.73m}^2$ , elevation of serum uric acid was independently associated with the occurrence of metabolic syndrome ( $\text{OR}[95\% \text{CI}]: 1.004[1.002-1.007]$ ) (Table 1) and NAFLD ( $\text{OR}[95\% \text{CI}]: 1.005[1.002-1.007]$ ), but not in those with renal insufficiency ( $\text{GFR} < 90 \text{ ml/min/1.73m}^2$ ). ③ By using new ultrasound quantitative method for hepatic fat content, we found that uric acid level was independently associated with hepatic fat content ( $P=0.003$ ) only in participants with normal renal function, but not in subjects with  $\text{GFR} < 90 \text{ ml/min/1.73m}^2$  (Table 1).

**Conclusion:** Hyperuricaemia was independently associated with MS and NAFLD in people with normal renal excretion function, but in patients with renal excretion insufficiency, the meaning of hyperuricaemia on metabolic disorders should be explained with caution.

Multivariate regression analysis showing factors independently associated with MS/hepatic fat content					
Metabolic syndrome			Hepatic fat content		
Independent variables	OR (95% CI)	P value	Independent variables	Standardized $\beta$	P value
GFR $\geq 90 \text{ ml/min/1.73m}^2$			GFR $\geq 90 \text{ ml/min/1.73m}^2$		
age	1.027(1.007-1.048)	0.008	BMI	0.348	$<0.001$
BMI	1.404(1.311-1.504)	$<0.001$	Age	-0.121	0.001
Uric acid	1.004(1.002-1.007)	0.002	Triglycerides	0.113	0.002
GFR $< 90 \text{ ml/min/1.73m}^2$			GFR $< 90 \text{ ml/min/1.73m}^2$		
BMI	1.506(1.377-1.648)	$<0.001$	Uric acid	0.110	0.003
age	1.029(1.007-1.052)	0.011	BMI	0.397	$<0.001$
-	-	-	Triglycerides	0.140	0.001
-	-	-	Age	-0.096	0.027

The values included for analysis of MS risk factors were age, sex, BMI, smoking status, alcohol drinking, GFR and Uric acid, and all the above parameters, liver enzymes and MS components were included for analysis of independent factors associated with hepatic fat content.

Supported by: China National Key Technologies R&D program

## 376

### Relationships of visit-to-visit variability and time-to-effect in systolic blood pressure to the risks of nephropathy and retinopathy in type 2 diabetes

T. Takao<sup>1</sup>, T. Ide<sup>1</sup>, H. Yanagisawa<sup>2</sup>, M. Kikuchi<sup>1</sup>, S. Kawazu<sup>1</sup>, Y. Matsuyama<sup>3</sup>;

<sup>1</sup>Division of Diabetes and Metabolism, The Institute for Adult Diseases, Asahi Life Foundation, Tokyo, <sup>2</sup>Department of Public Health and Environmental Medicine, The Jikei University School of Medicine, Tokyo, <sup>3</sup>Department of Biostatistics School of Public Health, University of Tokyo, Japan.

**Background and aims:** Visit-to-visit variability in BP has been found to independently add to the mean BP in predicting the risk of diabetic nephropathy (DNp), but not retinopathy (DR), in type 1 diabetes. Lack of a BP legacy effect on cardiovascular events has been reported in type 2 diabetes (T2D). This study aimed to determine whether systolic BP (SBP) variability can predict progression of DNp and DR, independently of the mean SBP, in patients with T2D, and to evaluate the time-to-effect relationship between SBP control and the risks of DNp and DR.

**Materials and methods:** A total of 670 (553 men, 117 women) patients with T2D who first visited our hospital from 1995 to 1996 with less than one year of discontinuation were followed up through 2010. Their mean age, duration of diabetes, BMI, BP, HbA1c, creatinine (Cr) and nonHDLcholesterol (nonHDL-C) at baseline were 55.8 years, 5.7 years, 23.4 kg/m<sup>2</sup>, 133.6/77.8 mmHg, 7.6%, 0.80 mg/dl and 160.6 mg/dl, respectively. Insulin therapy and antihypertensive drugs at baseline were administered to 86 and 158 patients, respectively. The total number of SBP measurements was 51,615. Progression of DNp was defined as doubling of Cr, and DR was onset of mild-moderate non-proliferative DR. 536 of 670 had no DR at first visit. 665 of 670 and 531 of 536 had at least two SBP determinations for each endpoint. SD or CV was used as a measure of SBP variability.

**Results:** By the end of follow-up, DNp had progressed in 31 patients and DR in 106. The SD or CV of SBP was a significant predictor of DNp, but not DR, independently of mean SBP (Table). SBP, analyzed as a time-dependent covariate (last observation carried forward, LOCF), was non-significant for DNp but significant for DR, after adjusting for the baseline covariates and mean of BMI, HbA1c and nonHDL-C. Adjusted HR for the annual mean SBP before progression of DNp and DR was obtained using the moving-mean method. HR of the mean SBP for DNp became significant during the 6-year period and gradually increased over the 15-year period before progression of DNp. HR of the mean SBP for DR was highest during the first year, then decreased and became non-significant during the 6-year period, and plateaued and decreased slowly during the 12- to over 15-year period before progression of DR.

**Conclusion:** Visit-to-visit SBP variability is a significant predictor of progression of DNp, but not DR, in patients with T2D, independently of the mean SBP. DNp progression was significantly associated with the mean SBP of more than 6 years earlier. In contrast, DR was less than 5 years earlier. These findings suggest a SBP legacy effect on progression of DNp, but not DR. SBP should be managed with emphasis on early and stable control to prevent DNp, and continuous lowering to prevent DR.

Multivariate Cox proportional hazard models for the progression of nephropathy and retinopathy							
	Nephropathy (n=665)			Retinopathy (n=531)			
	Model 1	Model 2		Model 1	Model 2		
	H R	H R		H R	H R		
	(95% CI)	(95% CI)	P-value	(95% CI)	(95% CI)	P-value	P-value
Mean SBP (10 mmHg)	1.64 (1.11-2.44)	2.10 (1.40-3.15)	0.014	1.24 (1.03-1.50)	1.21 (1.01-1.45)	0.023	0.039
SD SBP (mmHg)	1.25 (1.11-1.41)	–	0.0002	0.97 (0.89-1.05)	–	0.43	–
CV SBP	–	1.37 (1.17-1.62)	0.0001	–	0.96 (0.86-1.07)	–	0.49
Duration of diabetes (5 years)	1.37 (1.07-1.76)	1.37 (1.07-1.76)	0.012	1.22 (1.03-1.44)	1.22 (1.04-1.44)	0.018	0.018
Mean HbA1c (%)	0.85 (0.53-1.36)	0.85 (0.53-1.36)	0.50	1.94 (1.48-2.55)	1.94 (1.48-2.55)	<0.0001	<0.0001
Mean nonHDL-C (10 mg/dl)	1.28 (1.14-1.44)	1.28 (1.14-1.44)	<0.0001	1.02 (0.95-1.10)	1.02 (0.95-1.10)	0.53	0.54

Models were adjusted for age, sex, mean BMI, insulin therapy and antihypertensive drugs at baseline.

### 377

#### Glycaemic threshold for diabetes specific retinopathy among individuals from Saudi Arabia, Algeria and Portugal are not different from other populations

T.P. Almdal<sup>1</sup>, L.N. Handlos<sup>1</sup>, E. Juul<sup>1</sup>, M. Amtoft<sup>1</sup>, D. Vistisen<sup>1</sup>, L.B. Nielsen<sup>2</sup>, S.E. Badawi<sup>3</sup>, A.R.A. Sheikh<sup>4</sup>, M. Belhadj<sup>5</sup>, D. Nadir<sup>6</sup>, S. Zinai<sup>7</sup>, J. Raposo<sup>8</sup>, M. Kozarzewski<sup>9</sup>, H. Lund-Andersen<sup>1</sup>, D.R. Witte<sup>1</sup>;

<sup>1</sup>Steno Diabetes Center, Gentofte, Denmark, <sup>2</sup>Novo Nordisk, Bagsvaerd, Denmark, <sup>3</sup>UAE Ministry of Health, Dubai, United Arab Emirates, <sup>4</sup>King Abdul Aziz University Hospital, Jeddah, Saudi Arabia, <sup>5</sup>EHU, Oran, Algeria, <sup>6</sup>Algerian Ministry of Health, Algiers, Algeria, <sup>7</sup>Novo Nordisk Algeria, Algiers, Algeria, <sup>8</sup>APDP, Lisbon, Portugal, <sup>9</sup>Novo Nordisk, Lisbon, Portugal.

**Background and aims:** Previous studies from the US, Australia and Asia included in the Detect 2 study reports a glycemic threshold for diabetes specific retinopathy of 6.3 - 6.7%. There are only one such study from the Middle East and none from Europe. The aim of the present study was to investigate the glycemic threshold of retinopathy in populations from Saudi Arabia, Algeria and Portugal.

**Materials and methods:** Individuals without any previous history of diabetes was offered screening for diabetes in three countries; Saudi Arabia, Algeria and Portugal in an opportunistic design. All Individuals were offered determination of HbA<sub>1c</sub> (DCA 2000 technique). Among those with HbA<sub>1c</sub> <6.0% every 10<sup>th</sup> individual (n= 168) were offered retinal examination while all individuals (n= 582) with HbA<sub>1c</sub> ≥ 6.0% were offered retinal examination. The retinal examination consisted of 2 photos of the retina of each eye. The photos were assessed according to a modified ETDRS scale standard. Results are reported as presence of any retinopathy given abnormalities in a least one eye or presence of diabetes specific retinopathy in at least one eye.

**Results:** 789 individuals were screened; 299 in Saudi Arabia, 294 in Algeria and 196 in Portugal. Mean age varied from 44.6 years in Saudi Arabia to 60.8 years in Portugal and the proportion of men was between 46.1% and 54.0% in Portugal and Saudi Arabia, respectively. Mean HbA<sub>1c</sub> level was 6.6% in Saudi Arabia, 7.1% Algeria and 6.3% in Portugal, and BMI ranged from 29.4 kg/m<sup>2</sup> in Portugal to 31.6 kg/m<sup>2</sup> in Saudi Arabia. The prevalence of any retinopathy and diabetes specific retinopathy weighed according to probability of selection for retinopathy screening was 1.65% and 0.77%, respectively, in the sample from all three countries. The standardized risk for having retinopathy (for a 50-year old man) was 1.1% (0.6; 1.8) in Saudi Arabia, 1.9% (1.3; 3.0) in Algeria and 2.7% (1.7; 4.2) in Portugal.

**Conclusion:** The present study confirms that the glycemic threshold for diabetes specific retinopathy is HbA<sub>1c</sub> = 6.0 - 6.5%. Among persons with HbA<sub>1c</sub> ≥ 7% the prevalence is above 6% which also corroborates well with previous results.

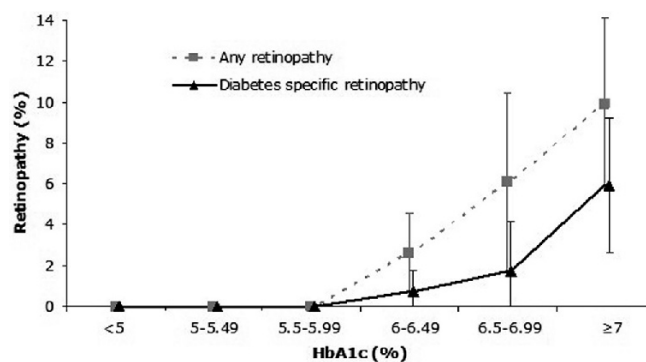


Figure 1. Prevalence of any retinopathy and diabetes specific retinopathy according to HbA<sub>1c</sub> group with 95% confidence intervals

Supported by: Novo Nordisk A/S



## PS 014 Beta cell GPCRs and other signalling mechanisms

378

### Islet GPR55 is coupled to increased insulin secretion and decreased apoptosis

S. Song<sup>1</sup>, B. Liu<sup>1</sup>, D. Baker<sup>2</sup>, G.C. Huang<sup>1</sup>, S.A. Amiel<sup>1</sup>, A.J. King<sup>1</sup>, J.E. Bowe<sup>1</sup>, P.M. Jones<sup>1</sup>, S.J. Persaud<sup>1</sup>;

<sup>1</sup>Diabetes Research Group, DNS Division, King's College London, <sup>2</sup>Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK.

**Background and aims:** We have recently reported that CB1 and CB2 cannabinoid receptors are expressed by islet  $\beta$ -cells where they regulate insulin secretion. A novel cannabinoid receptor, GPR55, is also activated by endocannabinoids, but it has some atypical features such as being activated by the CB1 antagonist AM251 and showing agonist activity to the lipid lysophosphatidylinositol (LPI). The physiological role of this receptor in islets has not been established so the current study investigated GPR55 expression and function in mouse and human islets.

**Materials and methods:** RT-PCR and Western blotting were used to detect GPR55 mRNA and protein expression, and insulin secretion from mouse and human islets was quantified by radioimmunoassay. Intraperitoneal glucose tolerance tests (IPGTTs) were performed on GPR55 knockout (KO) mice and their wildtype littermates. Apoptosis was induced by exposure of mouse islets to mixed cytokines for 20 hours and caspase-3/7 activities were quantified by a luminescent assay.

**Results:** Amplicons of the appropriate sizes were obtained using cDNAs from mouse and human islets with GPR55 primers, and an immunoreactive protein of the expected size was detected by Western blotting of mouse and human islet protein extracts. Activation of GPR55 using a pharmacological agonist (10  $\mu$ M O-1602) caused a reversible stimulation of insulin secretion from perfused mouse and human islets at 2mM glucose (peak stimulation, mouse:  $243 \pm 38\%$ ; human:  $450 \pm 200\%$ ,  $n=3-4$ ,  $P<0.05$ ). O-1602 significantly ( $P<0.05$ ) potentiated glucose-induced insulin secretion from mouse islets (peak stimulation above 20mM glucose plateau:  $220 \pm 44\%$ ,  $n=4$ ), and also from human islets (peak stimulation:  $190 \pm 29\%$ ,  $n=4$ ). In addition, the putative endogenous GPR55 ligand (5  $\mu$ M LPI) significantly elevated basal insulin secretion from mouse and human islets (peak stimulation, mouse:  $208 \pm 12\%$ ; human:  $1200 \pm 200\%$ ,  $n=3-4$ ,  $P<0.05$ ). Administration of LPI during the second phase of glucose-induced insulin secretion significantly potentiated insulin secretion from mouse and human islets (peak stimulation above 20mM glucose plateau, mouse:  $190 \pm 27\%$ ; human:  $747 \pm 180\%$ ,  $n=4$ ,  $P<0.05$ ). In static incubation secretion experiments a novel GPR55 agonist, CID1792197, also stimulated insulin secretion, as did AM251, a CB1 antagonist known to activate GPR55 in some cell types (2mM glucose:  $0.28 \pm 0.05$  ng/islet/h; + 10  $\mu$ M CID1792197:  $0.66 \pm 0.18$ ; + 10  $\mu$ M AM251:  $0.63 \pm 0.17$ ,  $n=6-8$ ,  $P<0.05$ ). IPGTTs of normal weight GPR55 KO mice indicated that there was a small impairment of glucose handling following GPR55 deletion (peak excursion at  $t=15$ , WT mice:  $11.4 \pm 0.9$  mM glucose; KO mice:  $14.1 \pm 1.7$  mM glucose,  $n=3-4$ ). Islets isolated from GPR55 KO mice also showed higher basal levels of apoptosis (luminescence units: WT mice:  $68,495 \pm 1,074$ ; KO mice:  $114,128 \pm 3,068$ ,  $n=16$ ,  $P<0.01$ ) and in the presence of cytokines (WT mice:  $253,047 \pm 7,125$ ; KO mice:  $283,403 \pm 6,678$ ,  $n=16$ ,  $P<0.01$ ).

**Conclusion:** GPR55 is expressed by islets and its activation with a range of pharmacological agonists stimulates insulin secretion from mouse and human islets *in vitro*. Global knockout of GPR55 was associated with a small decrease in glucose tolerance and enhanced islet apoptosis. These data are consistent with GPR55 playing an important role in glucose homeostasis through increased insulin secretion and maintenance of  $\beta$ -cell mass.

Supported by: King's College London, Diabetes UK, Henry Lester Trust, SCAST Trust

379

### CB<sub>1</sub> cannabinoid receptor ligands exhibit CB<sub>1</sub>-dependent and independent effects in CB<sub>1</sub> receptor-deficient mouse islets

C. Li<sup>1</sup>, J.E. Bowe<sup>2</sup>, D. Baker<sup>3</sup>, P.M. Jones<sup>2</sup>, S.J. Persaud<sup>2</sup>;

<sup>1</sup>Dasman Diabetes Institute, Kuwait City, Kuwait, <sup>2</sup>King's College London, UK, <sup>3</sup>Queen Mary University, London, UK.

**Background and aims:** We have previously demonstrated that islet CB<sub>1</sub> receptors are coupled to decreased cyclic AMP (cAMP) and increased intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ), and their activation with apparently selective pharmacological agonists stimulates insulin secretion from isolated mouse and human islets. However, both CB<sub>1</sub> agonists and antagonists have been reported to exert effects independently of their interaction with CB<sub>1</sub> receptors, so this study investigated the function of islets isolated from wildtype (WT) and CB<sub>1</sub> knockout (KO) mice and the effects of pharmacological cannabinoids on these islets.

**Materials and methods:** Islets were isolated from global CB<sub>1</sub> receptor-deficient mice and wildtype littermates by collagenase digestion of the pancreas. Cyclic AMP and  $[\text{Ca}^{2+}]_i$  levels were measured by ELISA and microfluorimetry, respectively. The dynamics and reversibility of insulin secretion were determined by radioimmunoassay following perfusion of isolated mouse islets *in vitro*.

**Results:** Islets isolated from KO and WT mice were indistinguishable in terms of yield and morphology. CB<sub>1</sub>-deficient islets exhibited enhanced insulin secretion in response to 20mM glucose ( $1,370 \pm 178\%$  vs  $699 \pm 76\%$  basal,  $n=4$ ,  $P<0.01$ ), which may be attributable, at least in part, to their higher insulin content (WT:  $30.8$  ng/islet; KO:  $50.5$  ng/islet). Administration of 10  $\mu$ M ACEA, a CB<sub>1</sub> agonist, significantly inhibited forskolin-induced cAMP production in WT islets ( $65 \pm 6\%$  of 10  $\mu$ M forskolin response,  $n=4$ ,  $P<0.05$ ), but had no effect in KO islets ( $91 \pm 16\%$  of 10  $\mu$ M forskolin response,  $n=5-7$ ,  $P>0.2$ ). Exposure of dispersed WT islet cells to 1  $\mu$ M ACEA led to increased  $[\text{Ca}^{2+}]_i$  ( $42 \pm 13\%$  of maximum tolbutamide response,  $n=3$ ), although subsequent treatment with 10  $\mu$ M ACEA elicited no response, most likely due to receptor desensitisation. However, 10  $\mu$ M ACEA unexpectedly caused a significant ( $P<0.01$ ) increase in  $[\text{Ca}^{2+}]_i$  in KO islets ( $39 \pm 6\%$  of maximum tolbutamide response,  $n=6$ ). ACEA activates TRPV<sub>1</sub> channels in other cells types and their activation is coupled to increased  $[\text{Ca}^{2+}]_i$  in  $\beta$ -cells, so the ACEA-mediated  $[\text{Ca}^{2+}]_i$  increase in CB<sub>1</sub>-deficient islet cells may occur via TRPV<sub>1</sub> activation. The competitive CB<sub>1</sub> antagonist AM251 significantly ( $P<0.05$ ) increased  $[\text{Ca}^{2+}]_i$  in WT islets ( $71 \pm 19\%$  of maximum tolbutamide response,  $n=4$ ), perhaps through activation of the novel cannabinoid GPR55 receptors, which we have identified in islets. As expected, 10  $\mu$ M ACEA reversibly potentiated insulin secretion from WT islets (from  $22.3 \pm 2.7$  pg/islet/min to  $35.1 \pm 2.2$ ,  $n=4$ ,  $P<0.01$ ), but it also exerted similar stimulatory effects in islets from KO mice (from  $33.6 \pm 3.2$  pg/islet/min to  $52.7 \pm 9.6$ ,  $n=4$ ,  $P<0.05$ ).

**Conclusion:** The use of islets isolated from CB<sub>1</sub> receptor knockout mice has demonstrated that ACEA and AM251 exhibit both CB<sub>1</sub> receptor-dependent and independent effects on  $[\text{Ca}^{2+}]_i$  and insulin secretion, suggesting that caution should be exercised when using these ligands to define the roles of CB<sub>1</sub> receptors in islet function.

Supported by: Diabetes UK

380

### Chronic activation of cannabinoid receptors modifies gene expression, glucagon content, but not insulin secretion from mouse islets *in vitro*

A. Vilches-Flores<sup>1,2</sup>, S.J. Persaud<sup>1</sup>;

<sup>1</sup>Diabetes Research Group, King's College London, UK, <sup>2</sup>Universidad Nacional Autonoma de Mexico, Mexico City, Mexico.

**Background and aims:** We have previously demonstrated that short term activation of cannabinoid receptors stimulates insulin secretion from mouse and human islets. There is evidence that the endocannabinoid system (ECS) is overactive in type 2 diabetes, which may impact on impaired glucose homeostasis. Islets produce endocannabinoids, but little is known about whether the local ECS may play a role in islet dysfunction. The aim of the current study, therefore, was to investigate the effect of chronic exposure to pharmacological cannabinoid receptors agonists on gene expression and secretory function of isolated mouse islets.

**Materials and methods:** Mouse islets were maintained in culture with 100nM ACEA (CB<sub>1</sub>R agonist) or 100nM JWH015 (CB<sub>2</sub>R agonist), for 48 hours and 7 days. Messenger RNAs encoding preproinsulin, preproglucagon, cannabinoid receptors (CB<sub>1</sub>R and CB<sub>2</sub>R), biosynthetic (NAPE-PLD, DAG-

L) and degrading (FAAH, MGL) enzymes were quantified by real-time PCR. Radioimmunoassay was used to determine dynamic insulin secretion from perfused islets, and islet insulin and glucagon contents.

**Results:** NAPE-PLD and FAAH mRNAs are relatively highly expressed in islets ( $0.35 \pm 0.002\%$  and  $2.1 \pm 0.1\%$  of preproinsulin mRNA levels, respectively), with much lower levels of CB1R, CB2R, DAG-L and MGL mRNAs (all  $<0.1\%$  of FAAH levels), suggesting that the synthesis and degradation of endogenous CB1R agonist is important in islet function. Islets treated with  $100\text{ nM}$  of the CB2R agonist JWH015 for 48 hours showed significant reductions in mRNAs encoding CB2R, DAG-L and NAPE-PLD (by  $54 \pm 16\%$ ,  $59 \pm 18\%$  and  $46 \pm 21\%$ , respectively,  $n=4$ ,  $P<0.01$ ), suggesting a negative feedback on receptor and biosynthetic enzyme expression. Exposure to JWH015 for 48 hours and 7 days led to significant reductions in preproglucagon mRNA (by  $70 \pm 10\%$ ,  $P<0.01$ ,  $n=3$  and  $36 \pm 5\%$ ,  $P<0.05$ ,  $n=4$ ), preproinsulin mRNA at 48 hours (by  $30 \pm 8\%$ ,  $P<0.05$ ,  $n=4$ ), and FAAH and MGL at 7 days (by  $42 \pm 5\%$  and  $37 \pm 2\%$ ,  $P<0.05$ ,  $n=4$ ). Glucagon content was also significantly reduced ( $45 \pm 15\%$ , and  $40 \pm 18\%$ ,  $P<0.05$ ,  $n=4$ ) following JWH015 exposure for 48 hours or 7 days and although there was an increase in insulin content this was not statistically significant ( $41.6 \pm 12.5$  ng/islet vs  $29.6 \pm 8.9$  in control,  $n=6$   $P>0.1$ ). These observations suggest that  $\alpha$ -cells could be more sensitive than  $\beta$ -cells to chronic activation of CB2 receptors. The CB1R agonist ACEA increased FAAH mRNA expression at 48 hours (by  $50 \pm 20\%$ ,  $n=4$ ,  $P<0.05$ ) and at 7 days ( $36 \pm 8\%$ ,  $n=4$ ,  $P<0.05$ ) of exposure, consistent with increased turnover of the endogenous CB1R agonist anandamide. Perfusion experiments showed that acute exposure to ACEA and JWH015 induced reversible increases in insulin secretion at  $3\text{ mM}$  glucose at 48 hours (by  $52 \pm 26\%$  and  $48 \pm 24\%$ ,  $n=3$ ,  $P<0.05$ ), and 7 days (by  $25 \pm 12\%$  and  $65 \pm 21\%$ ,  $n=3$ ,  $P<0.05$ ), both in control islets and in those treated chronically with cannabinoids. The  $16\text{ mM}$  glucose-induced insulin secretion was not modified in islets by chronic exposure to ACEA or JWH015.

**Conclusion:** These data indicate that chronic exposure of isolated mouse islets to a CB2R agonist has direct effects on gene expression of ECS components, on glucagon mRNA and protein content, but there are no deleterious effects on insulin content or insulin secretion. Chronic CB1R activation is associated with increased expression of the anandamide-degrading enzyme FAAH, but also does not disrupt glucose-induced insulin secretion.

*Supported by: Novo Nordisk UK, Diabetes UK*

## 381

### Expression and function of CCL5 and the receptors GPR75, CCR1, CCR3 and CCR5 in islets of Langerhans

Z. Hassan, B. Liu, S. Amisten, G.C. Huang, S.A. Amiel, P.M. Jones, S.J. Persaud;

Diabetes Research Group, King's College London, UK.

**Background and aims:** Chemokine ligand 5 (CCL5) is a 68 amino acid protein that belongs to the CC-chemokine subfamily, and stimulates the migration of monocytes and T cells to damaged and infected sites following an inflammatory insult. This is mediated via CCL5 activation of the CCR family of chemokine receptors. However, it is now apparent that CCL5 also stimulates the recently de-orphanised G-protein coupled receptor 75 (GPR75), which shows little sequence homology with the conventional chemokine receptors. CCL5 increases intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) in GPR75 transfected CHO cells and it also acts via GPR75 to inhibit neuronal cell death. The current study investigated the expression of CCL5, GPR75, CCR1, CCR3 and CCR5 in mouse and human islets of Langerhans, the effects of CCL5 on insulin secretion and the potentially protective effects of CCL5 activation of GPR75 on  $\beta$ -cell survival.

**Materials and methods:** CCL5, CCR1, CCR3, CCR5 and GPR75 expression and cellular localisation in mouse and human islets were examined by quantitative RT-PCR, western blotting and immunohistochemistry (IHC). Insulin secretion was quantified by radioimmunoassay after perfusion of isolated mouse islets. Apoptosis was induced by exposure of MIN6  $\beta$ -cells or mouse islets to mixed cytokines (IFN $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ ) or cis-diaminedichloroplatinum (cis-Pt) for 20 hours and caspase-3/7 activities were quantified by the luminescent CaspaseGlo assay. MIN6  $\beta$ -cell proliferation was quantified by measuring the rate of BrdU incorporation into cellular DNA.

**Results:** Quantitative RT-PCR indicated that islets expressed CCL5 and GPR75 mRNAs (CCL5:  $2.7 \pm 0.6\%$  and  $0.4 \pm 0.16\%$  of GAPDH mRNA expression, mouse and human islets respectively; GPR75:  $5.4 \pm 1.2\%$  and  $0.29 \pm 0.11\%$ , respectively,  $n=3$ ). CCR1, CCR3 and CCR5 mRNAs were also detected in mouse islets, but at significantly lower levels than quantified for GPR75, while CCR5 mRNA was not detected in human islets and only trace amounts of

CCR1 and CCR3 mRNAs were present. IHC revealed widespread GPR75 immunoreactivity in mouse and human islets, consistent with a  $\beta$ -cell localisation and a  $59\text{ kDa}$  immunoreactive protein was detected by western blotting. Islets also expressed the GPR75 ligand CCL5. Exposure of isolated mouse and human islets to exogenous CCL5 potentiated glucose-stimulated insulin secretion (mouse,  $20\text{ mM}$  glucose plateau:  $263 \pm 48\%$  basal;  $+20\text{ nM}$  CCL5:  $540 \pm 103\%$ ,  $n=4$ ,  $P<0.01$ ; human,  $20\text{ mM}$  glucose plateau:  $306 \pm 32\%$  basal;  $+10\text{ nM}$  CCL5:  $601 \pm 92\%$ ,  $n=4$ ,  $P<0.01$ ). In MIN6  $\beta$ -cells CCL5 significantly increased proliferation (BrdU incorporation, absence of CCL5:  $1.28 \pm 0.04$  absorbance units,  $+25\text{ nM}$  CCL5:  $1.71 \pm 0.10$ ,  $n=7$ ,  $P<0.01$ ) and reduced cytokine-induced caspase 3/7 activities (cytokines:  $1,073,086 \pm 24,422$ ;  $+25\text{ nM}$  CCL5:  $1,001,520 \pm 17,051$ ,  $n=8$ ,  $P<0.05$ ). CCL5 also significantly reduced apoptosis in mouse islets (cytokines:  $171,263 \pm 37,605$ ;  $+2.5\text{ nM}$  CCL5:  $76,395 \pm 8,573$ ; cis-Pt:  $78,210 \pm 8,370$ ;  $+2.5\text{ nM}$  CCL5:  $50,723 \pm 7,066$ ,  $n=8$ ,  $P<0.05$ ).

**Conclusion:** CCL5 is expressed by mouse and human islets where it may exert auto/paracrine effects via the most abundant CCL5 receptor, GPR75, to potentiate insulin secretion, stimulate  $\beta$ -cell proliferation and inhibit  $\beta$ -cell apoptosis. These data suggest that GPR75-selective agonists may be potential therapies for the treatment of Type 2 diabetes mellitus.

*Supported by: Diabetes UK*

## 382

### Regulation of $[\text{Ca}^{2+}]_i$ and insulin secretion by activation of FFAR1 in murine beta cells

G. Drews<sup>1</sup>, D. Cornejo<sup>1</sup>, A. Edalat<sup>1</sup>, G. Schulz-Raffelt<sup>2</sup>, M. Düfer<sup>1</sup>,

P. Krippeit-Drews<sup>1</sup>, E. Christiansen<sup>3</sup>, H.U. Häring<sup>2</sup>, T. Ulven<sup>3</sup>, S. Ullrich<sup>2</sup>;

<sup>1</sup>Pharmazeutisches Institut, Tübingen, Germany, <sup>2</sup>Department of Internal Medicine IV, University Hospital Tübingen, Germany, <sup>3</sup>University of Southern Denmark, Odense, Denmark.

**Background and aims:** Free fatty acids (FFAs) exert stimulating or inhibiting effects on beta cell function depending on the signalling pathways that are activated. The insulinotropic effect of FFAs is mediated by the stimulation of free fatty acid receptor 1 (FFAR1/GPR40), a Gq-coupled receptor which is proposed to activate phospholipase C. With respect to a possible insulinotropic action we aimed to investigate the effects of specific activation of FFAR1 by small receptor agonists and conjugated linoleic acids (CLAs) on glucose-induced insulin secretion (GIIS) and to analyse the changes of cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in primary mouse beta cells.

**Materials and methods:** In isolated islets and islet cells from C57BL/6 mice and FFAR1 knock-out mice insulin secretion was measured after static incubations by radioimmunoassay and  $[\text{Ca}^{2+}]_i$  by the fura-2 technique, respectively. FFAR1 was activated by a synthetic, small molecular agonist, TUG-469, and CLAs which do not stimulate FFAR2 and FFAR3 in expression systems.

**Results:** TUG-469 ( $10\text{ }\mu\text{M}$ ) augmented GIIS in wild type (from  $2.9 \pm 0.4$  to  $4.5 \pm 0.5\%$  of insulin content;  $n=11$ ;  $p \leq 0.05$ ) but not in FFAR1 knock-out mouse islets ( $2.1 \pm 0.3$  vs.  $2.5 \pm 0.3\%$ ;  $n=12$ , n.s.). Concomitantly, TUG-469 ( $100\text{ nM}$  and  $10\text{ }\mu\text{M}$ ) augmented  $[\text{Ca}^{2+}]_i$  at a stimulatory glucose concentration of  $15\text{ mM}$  ( $100\text{ nM}$ : from  $215 \pm 9\text{ nM}$  to  $284 \pm 17\text{ nM}$ ,  $n=11$ ,  $P \leq 0.01$ ;  $10\text{ }\mu\text{M}$ : from  $280 \pm 31\text{ nM}$  to  $355 \pm 27\text{ nM}$ ,  $n=17$ ,  $P \leq 0.05$ ). This effect was not owing to  $\text{Ca}^{2+}$  release from intracellular stores since the glucose- and TUG-induced increase in  $[\text{Ca}^{2+}]_i$  was abolished by the L-type  $\text{Ca}^{2+}$  channel blocker nifedipine ( $10\text{ }\mu\text{M}$ ) ( $n=11-18$ ). The SERCA inhibitor thapsigargin did not prevent the TUG-469-induced increase in  $[\text{Ca}^{2+}]_i$  ( $100\text{ nM}$  TUG: from  $194 \pm 17\text{ nM}$  to  $261 \pm 24\text{ nM}$ ,  $n=13$ ,  $P \leq 0.001$ ). Activation of muscarinic receptors by carbachol ( $10\text{ }\mu\text{M}$ ) still transiently increased  $[\text{Ca}^{2+}]_i$  from  $84 \pm 5\text{ nM}$  to  $196 \pm 20\text{ nM}$  after treatment of the cells with TUG-469 ( $n=18$ ,  $P \leq 0.001$ ). The effect of TUG-469 on  $[\text{Ca}^{2+}]_i$  was absent in beta cells of FFAR1 knock-out mice ( $n=20$ ). Similar to TUG-469, CLAs ( $100\text{ }\mu\text{M}$ ), widely used as dietary supplements, stimulated insulin secretion through FFAR1. The increase in  $[\text{Ca}^{2+}]_i$  induced by both isomers ( $100\text{ }\mu\text{M}$ ), CLA9c,11t (from  $265 \pm 21\text{ nM}$  to  $386 \pm 24\text{ nM}$ ,  $n=31$ ,  $P \leq 0.001$ ) and CLA10t,12c (from  $239 \pm 14\text{ nM}$  to  $340 \pm 20\text{ nM}$ ,  $n=32$ ,  $P \leq 0.001$ ), was, however, only partly reduced in  $\beta$ -cells of FFAR1 knock-out mice ( $n=31$  and  $n=46$ ,  $P \leq 0.05$  FFAR1 knock-out vs. wild type mice).

**Conclusion:** The specific activation of FFAR1 augments GIIS which makes FFAR1 attractive as a target for oral antidiabetic drugs. The insulinotropic effect of TUG-469 is solely due to FFAR1 activation while CLAs evidently exert additional effects on beta cell stimulus-secretion coupling. The data further suggest that FFAR1-induced IP $_3$ -dependent  $\text{Ca}^{2+}$ -release does not explain TUG-469 and CLA-induced changes in  $[\text{Ca}^{2+}]_i$ .

*Supported by: DZD and DFG*

## 383

**Purinoreceptor-mediated feedback in beta cells promotes diacylglycerol spiking, local protein kinase C activation and insulin secretion**

A. Wuttke, A. Tengholm;

Medical Cell Biology, Uppsala University, Sweden.

**Background and aims:** The lipid diacylglycerol (DAG) is generated in the  $\beta$ -cell plasma membrane in response to receptor and nutrient stimuli and plays an important role in various signaling processes. DAG facilitates membrane recruitment and activation of C1-domain-containing proteins like protein kinase C (PKC), which in turn amplifies insulin secretion. We recently discovered that glucose and depolarizing stimuli evoke pronounced DAG spiking in the plasma membrane of  $\beta$ -cells. The effect is mediated by ATP released from the secretory granules acting in an autocrine manner on purinoreceptors to activate phospholipase C. We now investigated whether these short-lasting DAG-transients are able to activate PKC and if they influence insulin secretion.

**Materials and methods:** Various fluorescent translocation biosensors were expressed in MIN6  $\beta$ -cells or in primary mouse and human islet cells. DAG was measured with the two adjacent C1 domains of rat protein kinase Cy (C1aC1b-GFP or C1aC1b-mCherry), which translocate to the plasma membrane upon DAG formation. PKC-activity was detected with myristoylated alanine-rich C-kinase substrate (MARCKS-GFP), which dissociates from the plasma membrane upon phosphorylation by PKC. Insulin secretion kinetics was recorded with general receptor for phosphoinositides-1 (Grp1-4GFP), which detects phosphatidylinositol (3,4,5)-trisphosphate formation following autocrine insulin receptor activation.

**Results:** Glucose induced pronounced spikes (4–18 s duration,  $n=46$ ) of the plasma membrane DAG-concentration in MIN6 cells, primary mouse  $\beta$ -cells and human islet cells. Recordings from glucose-stimulated MIN6 cells co-expressing C1aC1b-mCherry and MARCKS-GFP showed that isolated DAG-spikes translated into transient PKC activation in spatially restricted regions of the plasma membrane. MARCKS-GFP dissociated from the membrane within 1 s after the increase in DAG, but MARCKS-reassociation was much slower than the duration of the DAG transient. Consequently, high frequency DAG-spiking tended to cause sustained MARCKS-GFP dissociation from the plasma membrane. The glucose-induced MARCKS-GFP dissociation, but not DAG spiking, was prevented by 0.5  $\mu$ M of the PKC-inhibitor staurosporine. A variant of MARCKS-GFP with mutated PKC phosphorylation sites failed to dissociate from the plasma membrane in response to glucose. Both DAG spiking and PKC activity was abolished by 10  $\mu$ M of the purinergic P2Y1 receptor antagonist MRS 2179, consistent with the involvement of feedback from exocytotically released ATP. Interestingly, MRS 2179 also reduced glucose-stimulated insulin secretion. Thus, the purinoreceptor inhibitor decreased both the initial amplitude of glucose-induced Grp1-4GFP translocation (reduction by  $41 \pm 10\%$ ,  $n=8$ ) and the area under the curve of the subsequent Grp1-4GFP oscillations (reduction by  $66 \pm 5\%$ ,  $n=10$  cells).

**Conclusion:** Glucose triggers spiking of the DAG-concentration in spatially restricted regions of the  $\beta$ -cell plasma membrane. This pattern translates into local and transient activation of PKC. The underlying feedback activation of purinergic receptors by exocytotically released ATP is important for appropriate control of insulin secretion.

*Supported by: EFSD/MSD grant and the Novo Nordisk Foundation*

## 384

**The role of klotho in pancreatic beta cells**

S. Schinner, B. Funk, F. Ülgen, W.A. Scherbaum;

Endocrinology, University Hospital Düsseldorf, Germany.

**Background and aims:** Klotho is a regulator of life-span and aging. Klotho deficient mice (“klotho mice”) show reduced insulin levels and increased insulin sensitivity. The role of klotho in pancreatic beta-cells is not known. Therefore, we have investigated the expression and function of klotho in beta-cells.

**Materials and methods:** Transient transfections of Ins-1 beta-cells. Isolation of primary mouse islets. Quantitative PCR and Western blots of islets of wild-type and klotho mice. Immunofluorescence. Proliferation assays.

**Results:** Klotho is expressed in pancreatic beta-cells of wild-type mice as assessed by immunofluorescence (co-localisation). The expression of klotho in pancreatic islets was confirmed on the RNA (quantitative PCR) and protein level (Western blots). In Ins-1 beta-cells exogenous klotho (100ng/ml) regulated the proliferation after chronic stimulation with Wnt3a: In contrast

to acute stimulation, chronic Wnt-stimulation inhibited cell proliferation by 80%. This effect was completely reversed by simultaneous treatment with recombinant klotho. Mechanistically, the activation of canonical Wnt-signaling (assessed by a Topflash reporter gene) by Wnt3a was inhibited by additional klotho protein.

**Conclusion:** Klotho is a novel protein expressed in pancreatic beta-cells. In vivo, klotho deficient mice show hypoplastic islets; in vitro klotho regulates beta-cell proliferation, suggesting klotho as a novel regulator of beta-cell function.

## 385

**ob/ob mouse islets exhibit reduced muscarinic M3 receptor expression and decreased secretory sensitivity to cholinergic receptor activation**

A.C. Hauge-Evans, C. Reers, Z. Tippu, S. Amisten, A. Vilches, S.J. Persaud, P.M. Jones;

Diabetes Research Group, King's College London, UK.

**Background and aims:** Islets are innervated by parasympathetic nerves which release acetylcholine (ACh) during feeding. In beta-cells the effect of ACh is mediated primarily via muscarinic M3 receptors (M3) leading to amplification of glucose-induced insulin secretion from the islets. Beta-cell specific knock-out of M3 results in reduced insulin secretion and impaired glucose tolerance so we therefore investigated the expression and function of M3 receptors in islets from a model of type 2 diabetes, the *ob/ob* mouse.

**Materials and methods:** M3 mRNA and protein expression was determined by qPCR and immunohistochemistry in islets isolated from 12-week old *ob/ob* and lean mice. Insulin content and secretion were assessed by radioimmunoassay and the latter normalized to protein content. Blood glucose levels were measured in non-fasted animals prior to islet isolation using a commercially available glucose meter.

**Results:** Non-fasting plasma blood glucose levels, average islet area and protein content were significantly increased in *ob/ob* mice (blood glucose:  $19.99 \pm 0.49$  mmol/l glucose vs.  $8.24 \pm 0.31$  mmol/l,  $p<0.01$ ,  $n=3$ ; area:  $84,195 \pm 7,678 \mu\text{m}^2$  vs.  $26,295 \pm 2,699 \mu\text{m}^2$ ,  $p<0.0001$ ,  $n=4$ ; protein content:  $1.84 \pm 0.19 \mu\text{g}/\text{islet}$  vs.  $1.20 \pm 0.09 \mu\text{g}/\text{islet}$ ,  $p<0.0001$ ,  $n=5$ ), whereas insulin content was unchanged (lean:  $77.76 \pm 11.04$  ng insulin/islet; *ob/ob*:  $62.64 \pm 5.78$  ng insulin/islet,  $p>0.2$ ,  $n=5$ ). M3 mRNA expression was decreased by 68% in *ob/ob* mouse islets compared to lean control islets (lean:  $0.97 \pm 0.09\%$  GAPDH expression, *ob/ob*:  $0.31 \pm 0.03\%$ ,  $p<0.005$ ,  $n=6$ ) and protein expression was reduced by 43% (lean:  $59.2 \pm 1.2\%$  total islet area, *ob/ob*:  $33.6 \pm 1.3\%$  total islet area,  $p<0.0001$ ,  $n=4$ ). Glucose-induced insulin secretion from *ob/ob* mouse islets was comparable to that of controls but their response to the acetylcholine analogue, carbamylcholine (CCh), was decreased by  $43 \pm 15\%$ . Thus, treatment with 0.5 mmol/l CCh resulted in a 4-fold increase in insulin secretion from control islets at 20mmol/l glucose ( $8.79 \pm 1.1$  vs.  $2.17 \pm 0.85$  ng insulin/ $\mu\text{g}$  protein  $p<0.05$ ,  $n=3$ ), whereas a 2.4-fold increase was observed in *ob/ob* mouse islets ( $5.02 \pm 1.12$  vs.  $2.12 \pm 0.36$  ng insulin/ $\mu\text{g}$  protein,  $p=0.09$ ,  $n=4$ ). To assess whether down-regulation of M3 in *ob/ob* mouse islets is linked to the specific genotype of the *ob/ob* mouse or is caused by the hyperglycaemic environment, M3 mRNA expression was assessed in islets from wild type ICR mice maintained in culture at 5.5 or 16 mmol/l glucose for 72h. Incubation at 16 mmol/l glucose significantly decreased M3 mRNA expression levels compared to those at 5.5 mmol/l (16 mmol/l:  $40 \pm 2\%$  time 0 control, 5.5 mmol/l:  $106 \pm 15\%$ ,  $p<0.05$ ,  $n=3$ ). Culture of islets in high glucose is reported to cause increased activation of protein kinase C (PKC) and this has been implicated in the down-regulation of GLP-1 receptors in *ob/ob* mouse islets. However, PKC-depletion by exposure to the PKC activator, PMA (0.5  $\mu\text{mol/l}$ ), for 72h did not increase M3 mRNA expression in islets from wild type ICR mice (106% control at 72h).

**Conclusion:** Parasympathetic regulation of insulin release is impaired in *ob/ob* mouse islets most likely due to reduced expression of M3 receptors. Our data suggest that the receptor down-regulation may be a PKC-independent consequence of the hyperglycaemic environment, and they imply that M3 receptors could be potential targets in the treatment of type 2 diabetes.

*Supported by: Diabetes UK*



## 386

**Glucose-induced metabolic memory in min6 is camkii dependent**

G.J. Santos, S.M. Ferreira, L.F. Rezende, A.C. Boschero;

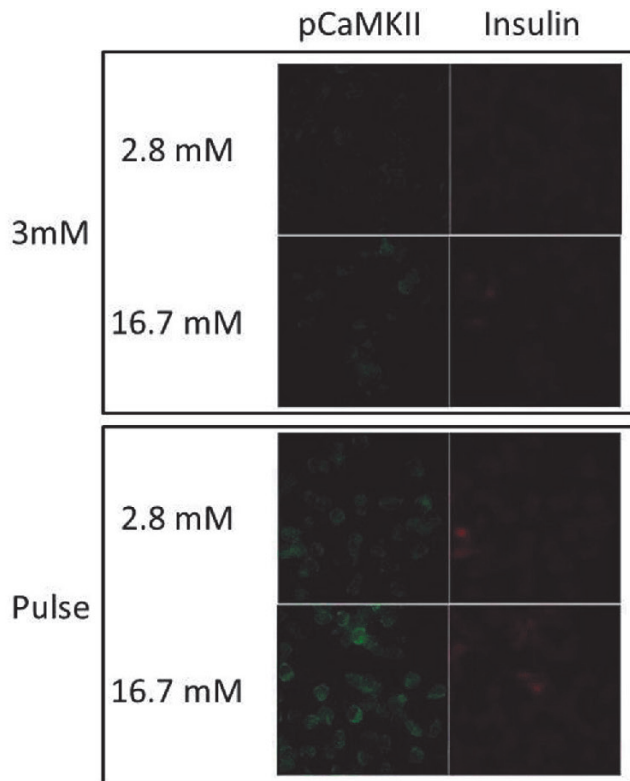
Department of Anatomy, Cell Biology and Physiology and Biophysics, State University of Campinas, Brazil.

**Background and aims:** Calcium-calmodulin kinase II (CaMKII), activated by the Ca<sup>2+</sup>-calmodulin complex, plays an important role in the Ca<sup>2+</sup>-signaling. In neurons, it is crucial for the generation of a process named Long Term Potentiation (LTP), responsible for formation of memory. It is also important for the process of neurotransmitters and hormones release. In this regard, CaMKII is increased in islets beta-cells during glucose-induced insulin secretion (GSIS), and animal lacking CaMKII shows a deficient glucose homeostasis. Therefore, we decided to evaluate if MIN6 cells possess the ability to form memory (acquire, store and retrieve information) in a CaMKII-dependent manner.

**Materials and methods:** MIN6 cells were exposed to 3 mM glucose (group 3mM) or to three pulses of 30 mM glucose of 1 h each, at intervals of 7 h (Group Pulse), with or without 10  $\mu$ M KN93 (a CaMKII specific inhibitor). After the stimulatory period, all the groups were maintained in a medium containing 3 mM glucose for 24 h (resting period). After, we assessed GSIS, CaMKII-phosphorylation, Calmodulin-pCaMKII association, and cell viability by RIA, Western Blot, Immunoprecipitation, and Flow cytometer, respectively.

**Results:** MIN6 cells secreted insulin in response to increasing concentrations of glucose, which correlated to increases with CaMKII phosphorylation and Calmodulin-pCaMKII interaction. Pulse treatment increased CaMKII phosphorylation and insulin secretion in both basal (2.8 mM) and stimulatory (16.7 mM) concentrations of glucose. Increase in both pCaMKII and insulin secretion over time (0–60'), even after removal of 16.7 mM glucose, were observed in the Pulse group. These alterations were not observed in the presence of KN93. In 3 mM group, there was an increase in Calmodulin-pCaMKII interaction, after exposure to 16.7 mM glucose, which was not observed in the Pulse group.

**Conclusion:** Even after 24 h, cells exposed to pulses of 30 mM glucose showed increased and prolonged GSIS, suggesting that MIN6 cells are able to acquire, store and retrieve information, that means to form memory. The inhibition of glucose-induced memory by KN93 indicates that CaMKII phosphorylation participates in this process.



**Immunofluorescence.** After 24 hours of the Pulse or 3mM treatment, MIN6 cells were exposed for 1 hour to 2.8 or 16.7 mM of glucose. After this period the cells were fixed and permeabilized, so, exposed, to an specific antibody.

Supported by: FAPESP, CAPES and CPq

## PS 015 Beta cell receptor tyrosine kinase and other signalling mechanisms

## 387

**Prevalent role of insulin receptor isoform A in insulin/IGF-I-induced beta cell hyperplasia**O. Escribano<sup>1,2</sup>, A. Gómez-Hernández<sup>1,2</sup>, C. Nevado<sup>1,2</sup>, G. García<sup>1,2</sup>,S. Fernández<sup>1,2</sup>, Y.F. Otero<sup>1,2</sup>, M. Benito<sup>1,2</sup>;<sup>1</sup>Biochemistry and Molecular Biology II, Complutense University of Madrid,<sup>2</sup>CIBERDEM, Barcelona, Spain.

**Background and aims:** Type 2 diabetes results from a combination of insulin resistance and impaired insulin secretion. While there is some debate about the primary defect in type 2 diabetes, insulin resistance is the most relevant pathophysiological feature in the prediabetic state. The main compensatory response to insulin resistance is the pancreatic beta cell hyperplasia to allow an increase in the insulin secretion. Previous work done in our laboratory showed a role of hepatic IGF-I in the compensatory beta cell hyperplasia observed in inducible liver-specific insulin receptor knockout mice (iLIRKO), a model of insulin resistance and type 2 diabetes. Moreover, several studies performed in pancreatic beta cells have shown differential insulin signaling depending on the type of activated insulin receptor isoform. However, the role of insulin receptor isoform A (IRA) or B (IRB) in the insulin/IGF-I-induced beta cell proliferation as well as glucose transport is poorly understood. Our aim was to generate a murine model of beta cells in order to study the role of insulin receptor isoforms in glucose transport, insulin/IGF-I-induced cell proliferation and also investigate the differential underlying mechanisms.

**Materials and methods:** Our laboratory had previously generated immortalized beta cell lines bearing IR (+/+), lacking IR (-/-), expressing exclusively IRA (IRA), or alternatively expressing IRB (IRB). In these cell lines, we measured the insulin/IGF-I-induced cell proliferation, glucose uptake and the underlying mechanisms involved were studied with standard biochemical techniques such as immunoprecipitation and Western blot.

**Results:** Cell proliferation studies have showed that cells expressing IRA are statistically more sensitive than those expressing IRB to the mitogenic response induced by insulin or IGF-I, being higher the mitogenic effect observed in response to IGF-I. Immunoprecipitation studies showed in IRA beta cells an enhanced Tyr-phosphorylation of IRS-1 in response to IGF-I and also an increased association of this protein with  $\alpha$ p85 compared to IRS-2. In summary, the main IGF-I signaling pathway occurred through IRA/IRS-1/ $\alpha$ p85/Akt/p70S6K. In the same way, IRA beta cells showed an increased glucose uptake as compared to control (+/+) and IRB beta cells under basal and insulin-stimulated conditions, this effect being likely owing to an enhanced association between the glucose transporters Glut-1 or Glut-2 with IRA and/or IGF-IR respectively.

**Conclusion:** Our results show a prevalent role of IRA in glucose uptake and IGF-I-induced pancreatic beta cell proliferation. These results could explain, at least partially, the molecular mechanisms involved in the compensatory beta cell hyperplasia in response to hepatic insulin resistance observed in iLIRKO mice.

Supported by: SAF2008/00031 and CIBERDEM (MICINN)

## 388

**Pkb/akt isoforms and the regulation of pancreatic beta cell mass and function**M.G. Dietrich<sup>1,2</sup>, R.A. Zuellig<sup>1</sup>, G.A. Spinas<sup>1,2</sup>, O. Tschopp<sup>1,2</sup>, M. Niessen<sup>1,2</sup>;<sup>1</sup>Endocrinology, Diabetes & Clinical Nutrition, University Hospital of Zurich, Switzerland, <sup>2</sup>Competence Center for Systems Physiology and Metabolic Diseases, Swiss Federal Institute of Technology (ETH) Zurich, Switzerland.

**Background and aims:** Protein kinase B (PKB/Akt) regulates growth, proliferation and survival in different cell types. Three isoforms exist (PKBa/Akt1; PKB $\beta$ /Akt2 and PKB $\gamma$ /Akt3) and all are expressed in pancreatic  $\beta$  cells. No alterations in islet phenotype after deletion of individual isoforms of PKB were described. We have previously presented evidence that PKBa regulates  $\beta$  cell growth, proliferation and survival in mice. The aim of this study is to

determine the regulation and function of the three isoforms of PKB in pancreatic  $\beta$  cells.

**Material and methods:** Pancreatic islets were isolated from male rats (Sprague-Dawley). Human islets were obtained from the Juvenile Diabetes Research Fund (JDRF) and the European Consortium for Islets Transplantation's (ECIT) "Islets for Research Distribution Program". Isoforms of PKB were overexpressed from adenoviral vectors. Glucose-induced insulin secretion (GSIS) was assessed by ELISA or RIA after incubation of rat or human islets, respectively, at 2.8 mM glucose and subsequent stimulation with 16.7 mM glucose. Proliferation was measured by bromodeoxyuridine (BrdU) incorporation.  $\beta$  Cell apoptosis was induced with IL-1 $\beta$  and determined by TUNEL staining. Growth factor-induced isoform-specific signalling and activation of isoforms was analysed by Western blotting.

**Results:** Overexpression of PKBa increased the rate of proliferation in rat islets 2.2 fold (in human islets: 4.1 fold). Overexpression of PKB $\beta$  did not significantly increase proliferation. Overexpression of PKBa and PKB $\beta$  increased  $\beta$  cell size to the same extent. IL-1 $\beta$  increased the rate of cell death in human islets more than 2 fold. Only overexpression of PKBa protected the islets from IL-1 $\beta$ -induced apoptosis. In rat islets overexpression of PKB $\beta$  increased insulin content 2.4 fold (in human islets: 1.15 fold) but it decreased GSIS (around 20%). Insulin content and GSIS was not significantly changed after overexpression of PKBa. IGF-I activated PKB in INS-1E cells around 10 fold more than insulin. IGF-I activated all three isoforms, but PKB $\beta$  activation was 2 fold higher compared to PKBa and PKB $\gamma$ . Insulin did not activate PKB $\gamma$  and activated PKB $\beta$  around 5 fold more compared to PKBa. Combined stimulation with insulin and IGF-I was not synergistic and altered the activation pattern of isoforms. Phosphorylation of GSK-3 was higher after stimulation with IGF-1 as compared to insulin. Phosphorylation of 4EBP-1 was increased by insulin but decreased by IGF-1. Stimulation with IGF-1 reduced phosphorylation of I $\kappa$ B $\alpha$ , but insulin increased it about 1.5 fold. Phosphorylation of JNK was lowered by insulin and IGF-1 to a similar extent (about 25%).

**Conclusion:** Although insulin and IGF-I share the same post receptor signal transduction cascades they activate PKB isoforms differently in INS-1E  $\beta$  cells. Similarly, insulin and IGF-I are not equivalent in regulating targets downstream of PKB. Our findings in human and rat pancreatic islets indicate that only PKBa regulates proliferation and apoptosis in pancreatic  $\beta$  cells. PKB $\beta$  might be involved in the regulation of insulin synthesis. Our results strongly indicate that PKB isoforms have specific and redundant functions in pancreatic  $\beta$  cells.

*Supported by: Olga Mayenfisch foundation, University of Zurich, Novo Nordisk*

## 389

**Impact of Akt1 and 2 on TBC1D1/D4 in response to glucose in beta cells** S. Rutti<sup>1</sup>, V. Kaddai<sup>2</sup>, F. Negro<sup>2</sup>, M. Kanzaki<sup>3</sup>, P.A. Halban<sup>1</sup>, K. Bouzakri<sup>1</sup>;

<sup>1</sup>Departement of Genetic Medicine and Development, University of Geneva, Switzerland, <sup>2</sup>Departement of Pathology and Immunology, University of Geneva, Switzerland, <sup>3</sup>Department of Biomedical Engineering, Tohoku University, Japan.

**Background and aims:** Protein kinase B/Akt plays a central role in beta-cells. The Rab-GAP AS160 (TBC1D4), an Akt substrate, is phosphorylated after glucose stimulation and downregulated in islets from type 2 diabetic individuals. However the impact of Akt isoforms on AS160 in response to glucose, as well as the expression and regulation in beta-cells of another Akt substrate, TBC1D1, remain to be investigated.

**Materials and methods:** Sorted rat primary beta-cells were transfected with siRNA to knockdown Akt1 and/or Akt2 (control = scrambled siRNA). Glucose stimulated insulin secretion (GSIS) was measured over 60 min. Proliferation (BrdU incorporation) and apoptosis (TUNEL) were assessed by immunofluorescence.

**Results:** Knockdown of Akt1 or Akt2 increased beta-cell apoptosis (Akt1:  $4.9 \pm 0.8\%$ , Akt2:  $2.4 \pm 0.17\%$  vs control:  $0.35 \pm 0.05\%$ ;  $p < 0.05$ ) and decreased proliferation (Akt1:  $1.49 \pm 0.14\%$ , Akt2:  $1.87 \pm 0.11\%$  vs control:  $10.22 \pm 0.3\%$ ;  $p < 0.05$ ), whereas only Akt1 knockdown decreased GSIS to  $0.99 \pm 0.31\%$  of total insulin content/h at 16.7 mM glucose, from  $2.3 \pm 0.22\%$  (control;  $p < 0.05$ ) without changing basal secretion. Both Akt isoforms regulated AS160/TBC1D4 phosphorylation in response to glucose. Akt2 knockdown decreased threonine-642 phosphorylation (western blot band density  $1.27 \pm 0.18$  vs  $4.51 \pm 0.83$  in control (arbitrary units);  $p < 0.05$ ), whereas Akt1 silencing decreased serine-588 phosphorylation ( $1.41 \pm 0.32$  vs  $4.58 \pm 0.48$  in control (arbitrary units);  $p < 0.05$ ). TBC1D1 was expressed in primary beta cells and phosphorylated (at serine 231 and 237) in response to glucose. However,

knockdown of Akt1 and 2 failed to modulate TBC1D1 phosphorylation (at serine 231 and 237).

**Conclusion:** Both Akt isoforms are involved in beta cell survival and proliferation, but only Akt1, not Akt2, appears to be implicated in GSIS. Both Akt1 and 2 regulate AS160/TBC1D4 phosphorylation in response to glucose. Akt2 is specifically involved in threonine-642 phosphorylation, whereas serine-588 phosphorylation is Akt1 dependent. TBC1D1 is expressed in primary beta-cells and is phosphorylated in response to glucose but such phosphorylation is not Akt-dependent.

## 390

**Influence of the ubiquitin-proteasome-system on the glucose sensor glucokinase in pancreatic beta cells**

A. Hofmeister-Brix<sup>1</sup>, S. Lenzen<sup>1</sup>, S. Baltrusch<sup>2</sup>;

<sup>1</sup>Institute of Clinical Biochemistry, Hannover Medical School, <sup>2</sup>Institute of Medical Biochemistry and Molecular Biology, University of Rostock, Germany.

**Background and aims:** In pancreatic beta cells, glucokinase (GK) acts as a glucose sensor and catalyses the rate-limiting step for initiation of glucose-induced insulin secretion. GK is mainly regulated on the posttranslational level. The ubiquitin-proteasome-system (UPS) has a pivotal role for protein turnover. In a recent study *in vitro* polyubiquitination of glucokinase was shown. However, studies regarding the interaction of the UPS and glucokinase in mammalian cells are still missing. The aim of this study was to analyse the protein stability and lifetime of glucokinase under treatment with the proteasome inhibitor Z-Leu-Leu-Leu-al (MG132) in MIN6 beta cells.

**Materials and methods:** MIN6 and primary mouse beta cells were incubated with 10  $\mu$ M MG132 and/or 10  $\mu$ g/ml cycloheximide (CHX) for 12 h. Cell viability was analysed by MTT-test, protein expression by Western blotting and immunofluorescence analyses. Glucokinase containing aggregate depositions were detected using the ProteoStat Aggresome Detection Kit. Glucokinase enzyme activity was determined using an enzyme coupled photometric assay. Insulin secretion was analysed by radioimmunoassay after stimulation with 3 or 25 mM glucose.

**Results:** Basal insulin secretion (3 mM glucose) and stimulated insulin secretion (25 mM glucose) were completely lost in MIN6 cells after a 12 h incubation with the proteasome inhibitor MG132 or CHX, an inhibitor of translation, alone or in combination. Cell viability was decreased by 30% under all experimental conditions. Glucokinase activity was reduced by 50% after treatment with MG132. However, the GK protein content increased as determined by Western blot analyses. In addition a second line with GK immunoreactivity was observed. After co-treatment with CHX this line was absent and GK activity was comparable to control conditions. Immunofluorescence analyses revealed that treatment of MIN6 and primary mouse beta cells with MG132 lead to an aggregation of GK in the cytoplasm. Such aggresomes were not observed in untreated cells and could be reduced by co-treatment with CHX.

**Conclusion:** Inhibition of the UPS for 12 h led to a total loss of glucose-induced insulin secretion in MIN6 cells, which could not be explained by the determined reduction in viability. The aggregation of GK in the cytosol after treatment with MG132 indicates that the specific protein lifetime is exceeded which results in protein missfolding and consequently in reduction of GK activity. Simultaneous inhibition of the translation with CHX increased the availability of long living chaperones which counteract this missfolding. This study points out the importance of the UPS for proper lifetime regulation of the glucose sensor GK in beta cells. Dysfunction of the UPS might contribute to the loss of glucose responsiveness in type 2 diabetes mellitus.

## 391

**The dual leucine zipper bearing kinase (DLK) levels are required for beta cell tasks by modulating PDX-1 activity and are impaired in human diabetic islets**N. Beeler<sup>1</sup>, B. Lefebvre<sup>2</sup>, R. Blouin<sup>3</sup>, P. Froguel<sup>4</sup>, F. Pattou<sup>2</sup>, G. Waeber<sup>1</sup>, A. Abderrahmani<sup>4,1</sup>;<sup>1</sup>Service of Internal Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland, <sup>2</sup>Department of Endocrine Surgery, Lille University Hospital; European Genomic Institute for Diabetes, University of Lille, France, <sup>3</sup>Département de Biologie, Faculté des sciences, Université de Sherbrooke, Canada, <sup>4</sup>University of Lille, European Genomic Institute for Diabetes, Lille, France.

**Background and aims:** Pro-inflammatory cytokines play a critical role in decline of pancreatic b-cells and thereby in development of diabetes. One of the mechanisms triggered by cytokines involves activation of the mitogen activated protein kinase (MAPK) also called c-Jun N terminal kinase (JNK) pathway. Activation of JNK is achieved through a canonical signaling cascade involving the MAP Kinase Kinase Kinases (MAP3Ks) and MAP2Ks, which finally culminates to phosphorylation of JNK. The goal of this project was to determine the role of the Dual Leucine Zipper Bearing Kinase (DLK) as the MAP3K of JNK signaling and in b-cells tasks.

**Materials and methods:** DLK expression was either increased by overexpression of plasmids coding for DLK or decreased by RNA interference in isolated islets from rat or in the insulin producing INS-1E and MIN6 cell lines. mRNA and protein levels were assessed by quantitative real-time PCR and western blotting respectively. Apoptosis has been quantified by counting pyknotic nuclei. PDX-1 binding activity has been measured by electrophoretic mobility shift assay (EMSA).

**Results:** We first tested the presence of DLK in  $\beta$ -cells. DLK is expressed in pancreatic  $\beta$ -cells and in islets whereas it is undetectable in other cell types. This selective expression pattern of DLK results from the absence of the REST/NRSF transcription factor. Silencing of DLK decreases the cytokine-induced activation of JNK and this is associated with a reduced insulin expression and an increase in cells death evoked by pro-inflammatory cytokines. Reduction of DLK diminished the PDX-1 binding activity. On the opposite, overexpression of DLK promotes insulin production and increases PDX-1 binding activity. Expression of DLK is reduced in islets from individuals with diabetes.

**Conclusion:** DLK regulates the JNK signaling and is required for b-cell tasks by maintaining PDX-1 activity.

*Supported by: The SNF, Chair of Excellence ANR N°ANR-10-CEXC-0*

## 392

**Heparan sulfate in beta cells: does it play a role in FGF signalling?**A. Theodoraki<sup>1</sup>, Y. Hu<sup>1</sup>, S. Poopalasundaram<sup>1</sup>, S. Guimond<sup>2</sup>, J. Turnbull<sup>2</sup>, P. Bouloux<sup>1</sup>;<sup>1</sup>Centre for Neuroendocrinology, Royal Free Hampstead NHS Trust, London, <sup>2</sup>Institute of Integrative Biology, The University of Liverpool, UK.

**Background and aims:** Heparan sulfate (HS) proteoglycans are ubiquitous macromolecules present on cell membranes and extracellular matrix. They modulate the binding of heparin-binding growth factors to their receptors regulating the transmission of signals to the intracellular compartment. Fibroblast growth factors (FGFs) consist of a family of structurally related heparin-binding polypeptides. The FGF19 subfamily (FGF19, 21, 23) members have a weak affinity toward HS allowing them to escape into circulation and function as hormones. FGF21 protects beta cells from glucolipotoxicity. The aim of this study was to characterize the participation of HS chains in FGF signaling in beta cells. Additionally, to investigate if the glycosaminoglycan heparin protects the beta cell lines INS1 and MIN6 from reactive oxygen species induced cell death in vitro.

**Materials and methods:** INS1 and MIN6 cells were incubated with FGF1 or FGF21 for 2min-6hrs and signaling cascades were studied by Western blotting. HeparinaseIII was used (0.25-2.5IU/ml) to cleave endogenous HS chains. Enzymatic cleavage was confirmed with the antibodies 3G10 (western blots) and 10E4 (immunocytochemistry). Oxidative damage in cultured cells was induced with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The mitochondrial membrane potential marker TMRE and the nucleic acid dead cell indicator ToPro3 were used in flow cytometry to identify healthy (TMRE+, ToPro-), early apoptotic (TMRE-, ToPro-) and dead cells (TMRE-, ToPro+). Porcine intestinal mucosal heparin (PIMH) was used at a concentration 5-250ug/ml.

**Results:** Timed signaling experiments with FGF1(10-100ng/ml) and FGF21(50-400ng/ml) identified the maximum ERK1/2 activation 5-10min post ligand addition; pAkt/tAkt ratio started to increase 10min post FGF1 or FGF21 addition. Cleavage of endogenous HS with heparinaseIII did not alter the FGF1 or FGF21 signaling cascade through ERK1/2. HeparinaseIII upregulated Akt phosphorylation ( $1.796 \pm 0.226$  after 2hr incubation with heparinaseIII 2.5IU/ml vs.  $0.903 \pm 0.09$  after incubation with vehicle control in MIN6). This was independent to FGF ligand addition and through the GSK3 $\beta$ , but not the mTOR pathway. Co-incubation with heparin did not inhibit signaling through Akt, while heparin alone had no effect. H<sub>2</sub>O<sub>2</sub> (250uM for 6hrs) reduced the number of INS1 TMRE positive cells from  $83.09\% \pm 3.97$  in control to  $22.49\% \pm 3.02$ . Heparin rescued a significant proportion of cells from oxidative damage: TMRE+ cells increased to  $41.19\% \pm 1.57$  ( $p < 0.005$ ) when H<sub>2</sub>O<sub>2</sub> and PIMH 50ug/ml were added concomitantly. TMRE+ cells were  $47.24\% \pm 1.53$  ( $p < 0.001$ ) when cells were pre-incubated with PIMH 50ug/ml for 24hrs followed by H<sub>2</sub>O<sub>2</sub> and PIMH 50ug/ml addition ( $n=4$ ).

**Conclusion:** Our results suggest that in the INS1 and MIN6 beta cells endogenous heparan sulfate chains are not indispensable for FGF signaling. HeparinaseIII upregulates the PI3K pathway. An important role of HS in beta cells seems to be the protection from reactive oxygen species induced cell death.

## 393

**Cross-talk between the c-Kit and insulin receptors enhance beta cell proliferation and function**

Z.C. Feng, A. Jan, J. Li, R. Wang;

Physiology &amp; Pharmacology, Children's Health Research Institute, the University of Western Ontario, London, Canada.

**Background and aims:** c-Kit, also known as a stem cell marker, is a member of the receptor tyrosine kinase superfamily. Expression of c-Kit is found on multiple tissues, including a subpopulation of beta cells. Interactions between c-Kit and its ligand, stem cell factor (SCF), play an integral role in the survival and function of beta cells, primarily via the PI3K/Akt/GSK3 $\beta$  signaling pathway. Our recent study demonstrated that mice with beta cell specific c-Kit overexpression had significant up-regulation of insulin receptor (IR) expression. Meanwhile, the contrary was observed in c-Kit loss-of-function (c-Kit<sup>Wv/+</sup>) mouse islets, suggesting that c-Kit modulates beta-cells by interacting with insulin signaling pathways. However, the underlining molecular mechanisms regarding this cross-talk have not been studied. The objective of the present study is to investigate the role of c-Kit with respect to IR and insulin receptor substrate (IRS) expression and the downstream signaling pathways implicated in modulating beta cell proliferation and function.

**Materials and methods:** INS-1 cells were cultured with either SCF or c-Kit siRNA. Expression and phosphorylation of c-Kit, IR, IRS, their downstream signaling pathways and islet transcriptional factors (e.g. Pdx1, MafA) was examined by qRT-PCR, western blot, co-immunoprecipitation, as well as double immunofluorescence assays.

**Results:** c-Kit and IR are both expressed in INS-1 cells. After SCF stimulation, we observed increased co-localization of c-Kit with IR as determined by double immunofluorescence and immunoprecipitation analyses. SCF-stimulated c-Kit and IRS1 phosphorylation occurred immediately (within 5 minutes) and reached peak level at 24 hours. Exogenous SCF treatment enhanced IR gene and protein expression ( $p < 0.05$ ), while significantly increasing pIRS1 ( $p < 0.01$ ) in INS-1 cells, and also lead to elevated Pdx-1 and MafA expression ( $p < 0.05$ ) when compared to controls. In line with these results, siRNA knock-down of c-Kit resulted in significantly decreased IR ( $p < 0.01$ ) and pIRS1 ( $p < 0.05$ ) protein levels with down-regulation of Pdx-1 and MafA expression. Furthermore, SCF/c-Kit-mediated improvements in cell proliferation, insulin, IR and pIRS1 expression were inhibited by wortmannin, indicating a requirement of the PI3K/Akt pathway.

**Conclusion:** These data suggest that c-Kit and IR may form a complex in INS-1 cells, and that cross-talk between these two receptors can enhance beta cell proliferation and function in a PI3K/Akt pathway-dependent manner. These data will advance our understanding of beta cell biology and enable us to develop better methods for the treatment of diabetes.

*Supported by: Canadian Institute of Health Research*



## 394

**Role of PTP1B in beta cell plasticity and function**R. Fernandez Ruiz<sup>1,2</sup>, E. Vieira<sup>1,2</sup>, P.M. García-Rovés<sup>2</sup>, R. Gomis<sup>1,2</sup>;<sup>1</sup>CIBERDEM, <sup>2</sup>IDIBAPS-Hospital Clinic, Barcelona, Spain.

**Background and aims:** Insulin resistance and  $\beta$  cell dysfunction are indispensable for the development of type 2 diabetes. In a prediabetic state,  $\beta$  cell mass and function are able to compensate for the diminished capacity of peripheral tissues to respond to insulin. Insulin has major autocrine effects on  $\beta$  cells, and in this sense, it is able to maintain both the glucose sensing machinery and promote  $\beta$  cell growth and survival. Protein tyrosine phosphatase 1B (PTP1B), a phosphotyrosine phosphatase anchored to the cytoplasmic face of the endoplasmic reticulum, inhibits insulin signalling through dephosphorylation of tyrosine residues in the insulin receptor and insulin receptor substrates. Due to the critical role that PTP1B plays regulating insulin action and its known effect in cell proliferation, our objective is to decipher the role of PTP1B in  $\beta$  cell mass and function.

**Materials and methods:** PTP1B knockout mice (KO) were kindly donated by Abbott Laboratories. At 8 weeks of age an intravenous glucose tolerance test (IPGTT) was performed by injecting 2g of glucose per Kg body weight, after an overnight fasting in knockout and wild type (WT) mice. Glycemia and insulin levels were measured from tail vein at different time points during the IGTT. Pancreas morphometry was performed by insulin and glucagon immunostaining. Studies of  $\beta$  cell proliferation and apoptosis were performed by ki67 and caspase3 immunostaining, respectively. Islets lysates were prepared in RIPA buffer for protein expression analysis.

**Results:** Whole body glucose tolerance was measured in WT and KO mice ( $n=13-14$ ) after a glucose load. Glucose levels are significantly lower in KO mice at 15, 30 and 60 minutes after glucose injection ( $p<0.05$ ), reflecting a higher glucose tolerance than in WT mice. The area under the curve is significantly lower in KO than in WT mice ( $23859.9\pm2544.58$  vs  $16282.9\pm2862.52$  mg\*min/dl). Insulin levels during the IPGTT shows an insulin secretion delay in KO mice versus WT. Insulin levels are significantly different ( $p<0.05$ ) both at 0, 15 and 60 minutes during the test between both groups. Analysis of  $\beta$  cell mass shows a significant increase ( $p<0.05$ ) in KO mice versus WT ( $81.87\pm11$  vs  $48.79\pm4$  ug/pancrea weight). In line with increased  $\beta$  cell mass a higher percentage of  $\beta$  cell proliferation ( $0.765\pm0.17$  vs  $0.072\pm0$ ), and a lower percentage of  $\beta$  cell apoptosis ( $1.37\pm0.0003$  vs  $4.58\pm0.0013$ ) was observed in KO versus WT mice. Moreover, these results of proliferation and apoptosis are in line with a higher Akt/PKB and Erk1/2, and a lower p53 protein expression in KO compared to WT mice. In order to assess the potential role of PTP1B in a pathological condition, we treated WT and KO mice with streptozotocin. After 7 weeks follow-up, the glycemia was significantly lower in KO mice, concomitantly with a higher  $\beta$  cell mass, higher  $\beta$  cell replication, and lower  $\beta$  cell apoptosis.

**Conclusion:** Ablation of PTP1B improves peripheral insulin sensitivity and increases  $\beta$  cell mass by mean of a higher  $\beta$  cell proliferation rate and a reduction in apoptosis. Thus, PTP1B is a potential therapeutic target for the treatment of diabetes due to improvements in insulin sensitivity and its positive actions in  $\beta$  cell plasticity and function.

*Supported by:* MICINN; Generalitat de Catalunya

## PS 016 Secretory granule and cytoskeleton dynamics in the beta cell

## 395

**Role of Rab3 family members in the regulation of beta cell insulin secretion**

C. Johné, S.E. Baltrusch;

Institute of Medical Biochemistry and Molecular Biology, University Rostock, Germany.

**Background and aims:** Rab proteins, that belong to the Ras superfamily are small GTPases with crucial functions in vesicle trafficking. Studies in the past decade revealed that Rab proteins, namely Rab27A and Rab3A, are also involved in insulin dense core vesicle trafficking and thus, impact insulin secretion from pancreatic beta cells. Rab27A has an effect on the replenishment of the docked granule pool and, on the coordination of pre-exocytotic stages. Rab3A interacts with different regulators promoting docking and exocytosis. Recent studies proposed that another member of the Rab family, Rab3B, has a pivotal function in beta cell insulin secretion. Therefore the aim of this study was to explore the role of Rab3B in beta cells.

**Materials and methods:** Mouse islets, murine tissues and MIN6 beta cells were analyzed for Rab3 expression by RT-PCR, Western Blot and immunofluorescence analyses. For overexpression of Rab3A and Rab3B, cells were transiently transfected. Down regulation of Rab proteins was evoked by lentiviral shRNA transduction. Glucose-induced insulin secretion was measured by ELISA.

**Results:** Both, Rab3A and Rab3B are expressed in mouse islets, brain, liver and pancreas as well as in MIN6 beta cells. In total pancreas tissue Rab3A was expressed 10-fold higher than Rab3B. In contrast, an equal amount of Rab3A and Rab3B was detectable in isolated islets, indicating the pivotal role of Rab3B in endocrine tissue. Immunofluorescence analyses revealed a punctate distribution of Rab3A and Rab3B in beta cells. Surprisingly, the observed Rab3A and Rab3B vesicle-like localization showed only little colocalization with each other. Rab3A displayed a higher degree of colocalization with insulin granules than Rab3B. Islets and MIN6 cells were cultured for 72 h in the presence of palmitate at high or low glucose concentrations. The expression of both, Rab3A and Rab3B decreased by palmitate incubation in MIN6 cells. However, a significant increase in the expression level of both proteins was observed in islets. Furthermore the impact of Rab3 proteins on insulin secretion was studied. Neither Rab3A nor Rab3B overexpression in MIN6 beta cells modified insulin secretion. In contrast, knocking down Rab3A or Rab3B protein expression resulted in a significant reduction of glucose-induced insulin secretion.

**Conclusion:** Our study confirmed the key role of Rab3A in regulation of murine beta cell insulin secretion. A recent publication suggested that rather Rab3B is the crucial regulator of insulin dense core vesicle trafficking in human islets. We could show for the first time in mouse islets Rab3B expression and alterations in the expression level by means of nutrient. Thus, Rab3B and Rab3A are crucial factors in beta cell function and reduced or imbalanced expression may contribute to progression of type 2 diabetes mellitus.

*Supported by:* DDG

## 396

**Glucose-induced ERK1/2 activation is permissive for receptor-operated potentiation of insulin secretion through cytoskeletal remodelling**

J.E. Bowe, A. Chander, S.J. Persaud, P.M. Jones;

Diabetes Research Group, King's College London, UK.

**Background and aims:** We have previously shown that several receptor-operated secretagogues, including kisspeptin, the cannabinoid 2 receptor agonist JWH015, the GLP-1 analogue exendin-4 and the CaR agonist A568, potentiate glucose-induced insulin release via an ERK1/2-dependent pathway. The present study aimed to further examine the mechanism by which ERK1/2 modulate receptor-operated potentiation of insulin release and to investigate whether actin dissociation might be involved.

**Materials and methods:** Isolated mouse islets were incubated for 1hr in a physiological salt solution and insulin release was measured by radioimmunoassay. Activation of ERK1/2 was measured by western blotting for phos-

pho-ERK relative to total ERK immunoreactivity. Dispersed mouse islet cells were incubated for 10 min in a physiological salt solution containing various treatments, before fixation in 4% paraformaldehyde and staining for actin filaments with rhodamine-phalloidin.

**Results:** Neither kisspeptin, A568 nor exendin-4 significantly stimulated insulin release from islets at a sub-stimulatory glucose concentration (2 mmol/L glucose control:  $0.14 \pm 0.02$  ng/islet/h, 1  $\mu$ mol/L kisspeptin:  $0.14 \pm 0.02$ , 10  $\mu$ mol/L A568:  $0.16 \pm 0.05$ , 100 nmol/L exendin-4:  $0.13 \pm 0.04$ ,  $n=9$ ,  $p>0.2$ ). However, in the presence of 10  $\mu$ mol/L okadaic acid and 100  $\mu$ mol/L sodium pervanadate, which activated ERK1/2 through inhibition of dephosphorylation, these secretagogues significantly increased insulin release from islets incubated at 2 mmol/L glucose (1  $\mu$ mol/L kisspeptin:  $381 \pm 52\%$  basal, 10  $\mu$ mol/L A568:  $456 \pm 39\%$ , 100 nmol/L exendin-4:  $322 \pm 30\%$ , 10  $\mu$ mol/L JWH015:  $210 \pm 19\%$ ,  $n=9$ ,  $p<0.05$ ). Dissociation of actin filaments by 1 h treatment of islets with either 10  $\mu$ mol/L cytochalasin-B or 10  $\mu$ mol/L latrunculin enabled all receptor-operated secretagogues tested to significantly stimulate insulin release from islets incubated at 2 mmol/L glucose (islets treated with 10  $\mu$ mol/L cytochalasin-B: 1  $\mu$ mol/L kisspeptin:  $315 \pm 32\%$  basal, 10  $\mu$ mol/L A568:  $407 \pm 35\%$ , 100 nmol/L exendin-4:  $351 \pm 24\%$ , 10  $\mu$ mol/L JWH015:  $192 \pm 29\%$ ,  $n=9$ ,  $p<0.05$ . Islets treated with 10  $\mu$ mol/L latrunculin: 1  $\mu$ mol/L kisspeptin:  $287 \pm 27\%$  basal, 10  $\mu$ mol/L A568:  $816 \pm 138\%$ , 100 nmol/L exendin-4:  $427 \pm 59\%$ , 10  $\mu$ mol/L JWH015:  $245 \pm 39\%$ ,  $n=9$ ,  $p<0.2$ ). Furthermore, a decrease in filamentous actin was observed in primary  $\beta$ -cells at 20 mmol/L glucose compared to  $\beta$ -cells incubated in the presence of 2 mmol/L glucose (2 mmol/L glucose: staining intensity  $56.9 \pm 9.3$  arbitrary units, 20 mmol/L glucose:  $24.2 \pm 4.4$ ,  $n=38-52$ ,  $p<0.05$ ) and this dissociation of actin filaments was reduced by the MEK inhibitor PD098059 (20 mmol/L glucose + 50  $\mu$ mol/L PD098059:  $39.5 \pm 4.1$  arbitrary units,  $n=49$ ,  $p<0.05$ ).

**Conclusion:** These observations suggest that the permissive role that ERK1/2 activation plays in the potentiation of glucose-induced insulin secretion by some receptor-operated agonists is mediated through actin dissociation.

Supported by: DRWF and Diabetes UK

## 397

### The synaptotagmin isoform 11 interacts with components of the RNA-induced silencing complex RISC at the ER-Golgi intermediate compartment

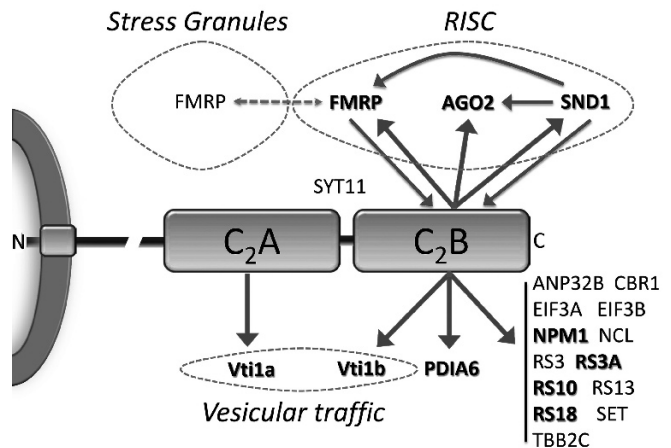
J. Lang<sup>1</sup>, A. Milochau<sup>1</sup>, V. Lagrèe<sup>1</sup>, S. Chaignepain<sup>1</sup>, A. Lajoix<sup>2</sup>, I. Garcia-Arcos<sup>3</sup>, C. Monterra<sup>1</sup>, L. Coudert<sup>1</sup>, J.-M. Schmitter<sup>1</sup>, B. Ochoa<sup>3</sup>,  
<sup>1</sup>Université de Bordeaux, Pessac, France, <sup>2</sup>Faculté de Pharmacie, Université Montpellier 1, France, <sup>3</sup>Department of Physiology, University of the Basque Country, Bilbao, Spain.

**Background and aims:** Synaptotagmins form a family of transmembrane proteins that contains two cytosolic  $C_2$  domains. Whereas some isoforms co-operatively bind calcium and membrane phospholipids via their  $C_2$  domains, others contain calcium-insensitive  $C_2$  domains. The function of several  $Ca^{2+}$ -insensitive isoforms have been characterized in detail in post-Golgi trafficking including exocytosis, where they act as regulators of membrane fusion. In contrast, the localization and the biological role of most other isoforms are still poorly understood. We have therefore addressed the localization and interactions of the calcium-insensitive isoform synaptotagmin 11 to obtain insight into its potential role.

**Materials and methods:** The polyclonal antibody against syt11 was produced by immunization of rabbits with the synthetic peptide CHQQAEEKKHK-TPPYK (amino acids 40–54 of rat syt11) coupled to KHL. Antibodies were further immunopurified. Islets, HIT-T15, MIN6 and INS-1E were cultured as previously published. Immunoprecipitations and pull-down experiments were performed on Triton X-100 extracts from INS-1E cells. Recombinant proteins were purified according to standard procedures. Peptide data were acquired on a MALDI Q-TOF Premier mass spectrometer used in MS/MS mode (Waters, Manchester, UK) and proteins were identified using the MASCOT software.

**Results:** In islets and clonal beta-cells synaptotagmin 11 localized in the early secretory pathway, at the ER/Golgi intermediate compartment (ERGIC) and in the cis-Golgi, as evidenced by subcellular fractionation and immunofluorescence. Novel binding partners of synaptotagmin 11 in INS-1E cells were identified by affinity binding assays coupled to mass spectrometry. A number of the binding partners identified here had been assigned previously to ER/Golgi derived-vesicles or ERGIC and a majority of them is linked to RNA synthesis, translation and processing. Whereas the  $C_2A$  domain of synaptotagmin 11 interacted with the Q-SNARE Vti1a, its  $C_2B$  domain interacted with the SN domains of the

staphylococcal nuclease domain-containing protein 1 (SND1), Ago2 and the fragile X mental retardation protein (FMRP), known components of the RNA-induced silencing complex (RISC). Upon induction of stress, only FMRP, but neither SND1 nor syt11 distributed with stress granules. **Conclusion:** Synaptotagmin 11 recruits RISC early in the secretory pathway via its  $C_2B$  domain and may provide a link between gene regulation by microRNAs and membrane traffic in the early secretory pathway.



Supported by: Université de Bordeaux 1 and exchange scholarship of the Basque Country

## 398

### ICA512 regulates the expression of villin-1, insights into the function of this actin binding protein in pancreatic beta cells

H. Mziaut<sup>1</sup>, D. Schumann<sup>2</sup>, T. Hildebrandt<sup>2</sup>, B. Mulligan<sup>1</sup>, A. Altkrüger<sup>1</sup>, C. Münster<sup>1</sup>, M. Lee<sup>3</sup>, S. Kauschke<sup>2</sup>, M. Mark<sup>2</sup>, P. Eickelmann<sup>2</sup>, M. Solimena<sup>1</sup>,  
<sup>1</sup>Molecular Diabetology, Medical School, University of Technology Dresden, Germany, <sup>2</sup>Cardiometabolic Research, Boehringer Ingelheim Pharma GmbH & Co. KGn, Biberach, Germany, <sup>3</sup>Department of Medicine, Samsung Medical Center, Seoul, Republic of Korea.

**Background and aims:** Insulin-containing secretory granules are formed at the trans-Golgi network and transported close to the plasma membrane for exocytosis. Stimulation of insulin secretion causes a rearrangement of microfilaments, but information about the interplay between insulin granules and cortical actin is still incomplete. Islet cell autoantigen 512/IA-2 (ICA512) is an intrinsic membrane protein of neurosecretory granules and a member of the receptor protein-tyrosine phosphatase family lacking phosphatase activity. Genetic deletion of ICA512 in mice results in mild glucose intolerance and decreased glucose-responsive insulin secretion. We have previously shown that ICA512 tethers insulin granules to actin microfilaments via its interaction with  $\beta 2$ -syntrophin. To gain further insights into how ICA512 regulates insulin secretion we have now compared the gene expression profiles of islets from ICA512<sup>-/-</sup> mice and control littermates.

**Materials and methods:** These studies included 2 groups of knockout mice, ICA512<sup>-/-</sup>, vil<sup>-/-</sup> and their control littermates, and insulinoma cell lines INS-1 and MIN6 cells. Statistical analyses were performed using the unpaired Student's t test.

**Results and Conclusion:** We report that ICA512<sup>-/-</sup> islets express significantly lower levels of villin-1, as first assessed by microarray assays and deep sequencing and then corroborated by immunocytochemistry and western blotting. Villin belongs to the gelsolin protein family and regulates the absorptive and secretory function of epithelial cells by modulating F-actin polymerisation/depolymerisation. Accordingly, we found that villin-1 is enriched at the cell cortex of pancreatic  $\beta$ -cells. As in mouse islets, downregulation of ICA512 in insulinoma Min6 and INS-1 cells by siRNA reduced villin expression. Furthermore, we found that villin regulates insulin secretion, presumably being required for proper changes in the organisation of the F-actin architecture. Preliminary evidence, in particular, suggests that downregulation of villin-1 in Min6 cells affects the processive movement of insulin granules. These results are consistent with the role of gelsolin-related proteins as regulators of exocytosis through their  $Ca^{2+}$ -dependent actin-bundling, -capping, and -severing activities. They also strengthen the close relationship between ICA512 and the cortical cytoskeleton. Specifically we propose that downregulation

lation of villin in ICA512-/- mice represents a compensatory adaptation to sustain the secretion of peptide hormones, including insulin.

Supported by: KKNDm

## 399

### Pre-exocytotic mobility of insulin granules in the submembrane space

K. Schumacher, K. Hatlapatka, M. Matz, K. Baumann, I. Rustenbeck;  
Technical University of Braunschweig, Germany.

**Background and aims:** Current hypotheses of insulin exocytosis provide that a pool of membrane-adjacent secretory granules exists which are in a primed and docked state and await one final trigger, a depolarization-induced influx of  $\text{Ca}^{2+}$ . This pool is held responsible for the first phase of glucose-induced insulin secretion. A number of recent observations have put this hypothesis into question.

**Materials and methods:** Granules in the immediate vicinity of the plasma membrane were visualized by transient transfection of insulin-secreting MIN6 cells with an insulin-EGFP fusion protein and imaged by TIRF microscopy. The cells were continuously perfused with HEPES-buffered Krebs-Ringer medium (37.0 °C), which was saturated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . The TIRF field had a calculated decay constant of 84 nm. The image files were evaluated by an in house written program (MATLAB 7.6.0) to achieve a complete observer-independent quantification. Granule mobility in the X/Y-plane was described by using the concept of the “caging diameter” (CD). The free cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) of MIN6 cells was measured with the Fura technique.

**Results:** Like primary mouse islets, MIN6 pseudo-islets responded to the depolarization by 40 mM KCl and the resulting increase of the  $[\text{Ca}^{2+}]_i$  with a strong increase in insulin secretion. In single MIN6 cells nearly 80% of the granules that were released by a perfusion with 40 mM  $\text{K}^+$  during 25 s acquisition sequences were already present in the first image of the sequence. The fastest itinerary took 3 s from granule arrival to exocytosis. Given that the residence time in the submembrane space prior to exocytosis was clearly different, and assuming that a certain pattern of mobility might precede exocytosis, the CDs of the last 3 s before fusion were calculated after normalizing the residence time to the time point of exocytosis. Granules that were released had initially a low CD value of about  $72 \pm 16$  nm which increased ( $p = 0.017$ , ANOVA) continuously to  $185 \pm 28$  nm at 0.125 s before fusion. Two types of control were considered. Firstly, the CDs of all those granules were determined that departed from the evanescent field in either direction and were present for 3 s or longer. Secondly, the CDs of a subgroup were determined, namely those granules that were the closest (both temporal and spatial) to the exocytoses. The former control had a CD value of about 140 nm and did not show a significant change ( $p = 0.324$ , ANOVA) in the 3 s time interval. However, the latter control which initially had also a CD value of about 140 nm showed a marginally significant increase ( $p = 0.078$ , ANOVA) to values of about 190 nm during the last 0.5 s.

**Conclusion:** Observer-independent quantification of the entire submembrane granule population by TIRF microscopy showed that pre-exocytotic granules significantly increased their mobility in the X/Y-plane during the last 3 seconds before fusion. This further calls into question the concept of a static membrane-attached granule pool as the correlate of the first phase secretory response.

Supported by: DDG, DFG, DDS

## 400

### Syntaxin recruitment to the release site marks the transition of insulin granules from tethered to docked

S. Barg, N. Gandasi;  
Medical Cell Biology, Uppsala University, Sweden.

**Background and aims:** The SNARE proteins syntaxin1A is required for both docking and exocytosis of insulin granules. Reduced expression of syntaxin is observed in type-2 diabetes and complete lack of syntaxin prevents granule docking in mice. Syntaxin clusters into raft-like nanodomains of up to 80 molecules in the plasma membrane, which may act as docking receptor for insulin granules. Here we tested two predictions of this model: syntaxin should be present at the release site before the granule docks, and association with a syntaxin cluster should mark a granule as docked and ready for exocytosis.

**Materials and methods:** Insulin-secreting Ins1 cells were co-transfected with syntaxin1a-EGFP and a granule marker and imaged using TIRF microscopy. **Results:** Syntaxin formed small clusters (<100nm) in the plasma membrane, often near docked granules. The clusters were stationary over several minutes, but exhibited striking intensity variations with a period of a few seconds. Single-molecule imaging showed that individual syntaxin molecules were mobile but occasionally captured near a granule, for a short time (<1s). Only granules associated with a syntaxin cluster remained stably docked over several minutes. Surprisingly, when a granule approached the plasma membrane it did not search for an empty syntaxin cluster. Instead, such clusters formed during granule docking at a rate of 0.6s-1. About two-thirds of the granules only visited the plasma membrane and did not stably dock; in these cases syntaxin did not accumulate. The rate at which visiting granules converted into stably docked was 0.1s-1. A syntaxin-derived peptide interfered with both cluster localization and prevented the transition of granules to stably docked. Finally, spontaneous loss of syntaxin clusters predicted subsequent loss of the granule by undocking.

**Conclusion:** We propose that visiting granules induce clustering of syntaxin in the target membrane, which then acts as their own docking receptor. This process marks the transition from loosely tethered to stably docked and occurs on a timescale consistent with rapid cellular signaling. It may thus underlie the docking defect observed with reduced expression of syntaxin.

Supported by: EFSD/MSD grant, VR, Diabetesfonden, Barndiabetesfonden, Novo Nordisk, Gustafsson

## 401

### Activated Cdc42-bound-IQGAP1 determines the cellular endocytic site in pancreatic beta cells

T. Kimura, M. Yamaoka, S. Taniguchi, I. Niki;  
Pharmacology, Oita University, Faculty of Medicine, Oita, Japan.

**Background and aims:** Intracellular protein and lipid trafficking is a fundamental cellular process that is required for the generation of specialized membranous organelles and for communication between these organelles. Recruitment of specific molecules to specific membrane sites is essential for this trafficking. Rab27a, a member of the Rab GTPase family, has been considered to act as a molecular switch for exocytosis in insulin secretion, cycling between an ‘active’ GTP-bound and an ‘inactive’ GDP-bound state. We previously showed that GDP-bound Rab27a is not an ‘inactive’ form but is in fact an ‘active’ form that regulates endocytosis through the GDP-dependent effector coronin 3. In the present study, we searched for another GDP-dependent Rab27a effector to investigate GDP-dependent roles of Rab27a in the pancreatic beta cell.

**Materials and methods:** Extracts from the insulin-secreting beta-cell line, MIN6, were applied to a GDP-bound Rab27a affinity column to investigate GDP-bound Rab27a interacting proteins. For immunostaining and live imaging, MIN6 cells were transfected with plasmid DNAs or siRNA using Lipofectamine 2000. After 24 h of transfection, the cells were analyzed with a confocal laser microscopy system. For the observation of endocytosis, cells were incubated in culture media containing the endocytosis marker. The rate of cells with cytoplasmic pattern was evaluated.

**Results:** We identified IQGAP1, an effector of GTP-bound Cdc42, as another GDP-dependent effector of Rab27a. We found that IQGAP1 interacted with GDP-bound Rab27a when it formed a complex with GTP-bound Cdc42. IQGAP1 regulated endocytosis of insulin secretory membranes. Silencing of IQGAP1 inhibited endocytosis and the glucose-induced redistribution of Rab27a and coronin 3 to the cell periphery. These processes could also be inhibited by disruption of the trimeric complex with dominant-negative IQGAP1 and Cdc42.

**Conclusion:** These results indicate that activation of Cdc42 in response to the insulin secretagogue glucose recruits endocytic machinery, including GDP-bound Rab27a and coronin 3, to IQGAP1 at the cell periphery and regulates endocytosis at this membrane site.

Supported by: KAKENHI



## 402

**Nonmuscle myosin II is involved in focal adhesion and actin remodelling controlling glucose-stimulated insulin secretion**

C. Arous, D. Rondas, P.A. Halban;

Dpt. Genetic Medicine and Development, University Medical Center, University of Geneva, Switzerland.

**Background and aims:** Regulation of insulin secretion from the beta cell is a key determinant of normal glucose homeostasis. Actin and focal adhesion (FA) remodelling, and activation of the ERK1/2 signalling pathway are essential for increased insulin exocytosis upon glucose stimulation. Nonmuscle myosin II isoforms may have a central role in glucose-stimulated insulin secretion (GSIS) because of their role in actin remodelling and FA maturation in other cell events. Furthermore, divergence of function between myosin IIa and IIb suggests possible isoform-specific roles in beta cells. Myosin light chain (MLCK) and Rho kinase (ROCK) are upstream regulators of myosin II that have been shown to be involved in GSIS. The aim was to elucidate the implication of myosin IIa and IIb in beta cell actin cytoskeleton remodelling, FA maturation and granule trafficking involved in GSIS.

**Materials and methods:** Three inhibitors were used to study the impact of myosin II regulation in GSIS: blebbistatin, specific inhibitor of myosin II; ML7, selective inhibitor of MLCK; Y23632, ROCK inhibitor. All studies (except live imaging) were performed using sorted rat primary beta cells cultured in monolayer, on extracellular matrix. After 2h at low glucose (2.8mM), cells were stimulated up to 60 min at high glucose (16.7mM). Insulin was measured by radioimmunoassay. Protein levels and phosphorylation were determined by western blot. Immunofluorescence and live cell imaging were used to study dynamic changes in localization and appearance of FA proteins, focal adhesion kinase (FAK) and paxillin (PAX), actin cytoskeleton and insulin granules. Live cell imaging was performed on MIN6B1 (mouse beta cell line) at 0mM (basal) and 20mM glucose (stimulated). Data are mean  $\pm$  SEM (n=3–4 independent experiments).

**Results:** Myosin II and MLCK inhibitors significantly decreased GSIS by 33.4 $\pm$ 5.2% and 25.5 $\pm$ 3.5% respectively whereas the ROCK inhibitor increased GSIS about 2-fold as previously shown. Glucose stimulation led to bundling of actin filaments, with FAK and PAX phosphorylation and their localization in small filopodial extensions resembling maturing FAs. ROCK inhibition caused disassembly of glucose-induced actin stress fibres in the central portion of the cell and resulted in large FAs containing pFAK and PAX without significant effect on FA number. MLCK inhibition was without apparent effect on actin remodelling induced by glucose but resulted in a 53.8 $\pm$ 3.3% decrease of ERK1/2 phosphorylation and 54.1 $\pm$ 5.1% decrease of FA number. Blebbistatin completely blunted actin reorganization and insulin granule movement, and decreased by 88.7 $\pm$ 6.0% FA protein recruitment to the membrane after glucose stimulation. Finally, glucose induced reorganization of both myosin IIa and IIb into fibres co-localizing with actin but not with PAX at filopodial extensions. Interestingly, only glucose-induced myosin IIa reorganisation was blunted by MLCK inhibition.

**Conclusion:** Myosin II seems to be directly involved in GSIS acting through actin cytoskeleton and FA remodelling and granule trafficking. Our results indicate an indirect role of myosin II on FA maturation and stabilization, likely through actin and/or microtubule regulation with MLCK and ROCK as upstream regulators. Finally, our data suggest a distinct regulation of FA formation induced by glucose through the MLCK-myosin IIa-ERK1/2 pathway.

Supported by: SNSF

## 403

**Potential role of Huntingtin-associated protein-1 in regulating insulin secretion from beta cells**G. Li<sup>1</sup>, B. Xie<sup>1</sup>, R. Luo<sup>1</sup>, X.-J. Li<sup>2</sup>, Y.-M. Bee<sup>3</sup>;<sup>1</sup>Dept of Clinical Research, Singapore General Hospital, Singapore, <sup>2</sup>Dept of Human Genetics, Emory University, Atlanta, USA, <sup>3</sup>Dept of Endocrinology, Singapore General Hospital, Singapore.

**Background and aims:** Huntingtin-associated protein-1 (HAP1) is involved in the pathogenesis of Huntington's disease. It may act as a scaffold in the assembly of protein complexes and participate in intracellular trafficking. There is evidence that HAP1 is expressed in pancreatic islets. We have detected HAP1 expression in insulin-secreting INS-1  $\beta$ -cells and thus investigated its potential roles by small RNA interference technology.

**Materials and methods:** INS-1 cells were transfected with a pool of siRNA oligos targeting HAP1 for 48–72h to knock down its expression as assessed by

qRT-PCR and immunoblotting. Subsequently, insulin secretion induced by glucose and other secretagogues was measured. The key events for stimulation of insulin secretion, including glucose metabolism, membrane potential depolarization and intracellular free  $\text{Ca}^{2+}$  levels ( $[\text{Ca}^{2+}]_i$ ), were also assessed in these cells.

**Results:** Transfection of HAP1-targeting siRNA oligos for 48–72h markedly knocked down HAP1 by >80% as assessed by qRT-PCR and immunoblotting in INS-1 cells. HAP1 knockdown significantly reduced glucose-stimulated insulin secretion up to 30%, without altering insulin content in INS-1 cells. However, the proximal signaling events of the signaling cascade implicated in regulating glucose-stimulated insulin secretion were not affected by HAP1 knockdown; these include the glucose metabolism, glucose-induced depolarization of membrane potential and elevation of  $[\text{Ca}^{2+}]_i$ . In addition, the effect of tolbutamide (an ATP-sensitive potassium channel blocker) on membrane potential depolarization remained intact, whereas its ability to stimulate insulin release was inhibited following HAP1 knockdown. Moreover, high (40 mM) KCl-induced insulin secretion was also significantly reduced in these cells, though  $[\text{Ca}^{2+}]_i$  elevation by high KCl was not affected. These results suggest that a step after  $[\text{Ca}^{2+}]_i$  rise in the insulin secretion scenario involves HAP1. On the other hand, glucose-stimulated insulin secretion was markedly potentiated by forskolin and IBMX which elevate cAMP level and activation of PKA. Interestingly, there was no significant change in glucose-stimulated insulin secretion in the presence of forskolin or IBMX after HAP1 knockdown in INS-1 cells.

**Conclusion:** All these results indicate that HAP1 knockdown interfered with glucose-stimulated insulin secretion beyond the steps of membrane depolarization and  $[\text{Ca}^{2+}]_i$  rise, and such inhibition could be relieved by cAMP elevation. Thus, HAP1 may participate in the regulating insulin secretion by involved in intracellular vesicles trafficking and exocytosis, such as motivating insulin-containing granules from reserved pool to docked pool in order to meet the need of rapid insulin release.

Supported by: National Medical Research Council of Singapore

## 404

**Proteomic approach to search for differentially expressed proteins between human pancreatic islets and human insulinomas**L.F. Terra<sup>1,2</sup>, P.C. Teixeira<sup>3</sup>, R.A.M. Wailemann<sup>1,2</sup>, A. Zelanis<sup>4</sup>, G. Palmisano<sup>5</sup>, E. Cunha-Neto<sup>3</sup>, J. Kalil<sup>3</sup>, L. Labriola<sup>1,2</sup>, M.C. Sogayar<sup>1,2</sup>;<sup>1</sup>Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil, <sup>2</sup>NUCEL, Universidade de São Paulo, Brazil, <sup>3</sup>InCor, FM-USP, São Paulo, Brazil, <sup>4</sup>Instituto Butantã, São Paulo, Brazil, <sup>5</sup>Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark.

**Background and aims:** Transplantation of isolated pancreatic islets is an alternative treatment for type 1 Diabetes; being limited by the shortage of organ donors. Ex-vivo culture prior to transplantation appears as an attractive alternative; however, maintenance of human islets in culture remains a difficult task. Only a few human beta-cell lines are available, therefore, culture of insulinomas, pancreatic tumors arising from beta-cells, may provide a tool to study cell proliferation and insulin synthesis and secretion. We have developed three human beta-cell lines (APM, VGA, CPR) which maintain the antigenic characteristics and insulin secretion profiles of the original tumors. In order to better characterize these beta cell lines, we set out to identify proteins displaying altered expression levels between normal and neoplastic beta cells.

**Materials and methods:** Identification of differentially expressed proteins was achieved through 2-dimensional gel electrophoresis (2D-DIGE) and mass spectrometry. Results were confirmed by western blotting.

**Results:** An average of 1,800 spots was detected; approximately 1% of which was differentially regulated. So far, we have been able to identify nine of these proteins, namely: a) Caldesmon, described as being involved in cytoskeletal organization, and NR6A1, which plays a putative role in germ cell development during gametogenesis, are both up-regulated in islets, when compared to the human insulinomas; b) MAGE-A2, described as being expressed in different tumors, but never in normal cells, 14-3-3 protein, whose classical role is to inhibit PKC and promote cell death inhibition, OTUD7A, whose putative function is presenting deubiquitinating activity, and Dermcidin, which has already been described as playing a role in cell migration and as a survival factor, were all found to be up-regulated in the APM cell line. None of these proteins had previously been described either in human insulinomas or in human islets; c) GAPDH, from the glycolytic pathway; PKN1, responsible for the regulation of microfilaments and NEUA, which catalyzes activation of the

N-acetylneuraminic acid, were also identified, but did not display differential expression.

**Conclusion:** Collectively, these observations not only prompt research towards successful establishment of bioengineered human beta-cells, providing a large and much needed source of cultured human beta-cell tissue for experimentation, but, could also lead to generation of therapeutic tools for insulinomas, since these differentially expressed proteins could be related to the malignancy displayed by these cells.

*Supported by: FAPESP, CNPq, PRP-USP*

## PS 017 Insulin secretion and exocytosis: novel aspects

### 405

**Ghrelin attenuates glucose-dependent ability of GLP-1 to increase cAMP, cytosolic  $\text{Ca}^{2+}$  and insulin release in islet beta cells**

**B. Damdindorj**<sup>1</sup>, K. Dezaki<sup>1</sup>, T. Kurashina<sup>1</sup>, R. Rita<sup>1</sup>, M. Kakei<sup>2</sup>, T. Yada<sup>1</sup>;

<sup>1</sup>Division of Integrative Physiology, Department of Physiology, Jichi Medical University, Tochigi, <sup>2</sup>First Department of Medicine, Saitama Medical Center, Jichi Medical University, Saitama, Japan.

**Background and aims:** A gastric hormone ghrelin and its receptor, growth hormone secretagogue-receptor (GHS-R), as well as ghrelin O-acyltransferase (GOAT), the enzyme that acylates the third serine residue of ghrelin, are all expressed in the pancreatic islets. Administration of GHS-R antagonists and GOAT inhibitors reportedly enhances plasma insulin responses and lowers glucose concentrations during glucose tolerance tests, indicating that ghrelin is a physiological insulinostatic hormone. We have reported that ghrelin stimulates pertussis toxin-sensitive  $\text{G}\alpha_{12}$ , an inhibitory subtype of GTP-binding proteins and attenuates cAMP signaling, thereby inhibiting glucose-induced increases in cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) and insulin release in islet  $\beta$ -cells. This study aimed to clarify whether exogenous ghrelin counteracts and blockade of endogenous ghrelin enhances effects of glucagon-like peptide-1 (GLP-1), a physiological incretin hormone, on insulin release, cAMP and  $[\text{Ca}^{2+}]_i$  signaling in rat islet  $\beta$ -cells.

**Materials and methods:** Islets of Langerhans were isolated by collagenase digestion from male Wistar rats. Insulin release and cAMP production in isolated islets were measured under static incubation and determined by ELISA.  $[\text{Ca}^{2+}]_i$  in isolated islets and single  $\beta$ -cells was measured by fura-2 microfluorimetry.

**Results:** In rat isolated islets under static incubation, glucose (8.3 mM)-induced insulin release was potentiated by 10 nM GLP-1, and this potentiation was suppressed by ghrelin at 10 nM. The glucose (8.3 mM)-induced cAMP production in islets was enhanced by GLP-1, and this enhancement was blocked by ghrelin. In the presence of 8.3 mM glucose, GLP-1 and an adenylate cyclase activator forskolin evoked  $[\text{Ca}^{2+}]_i$  increases in single  $\beta$ -cells and they were significantly attenuated by ghrelin. Blockade of the action of islet-derived ghrelin by [D-lys<sup>3</sup>]-GHRP-6, a GHS-R antagonist, enhanced glucose-induced insulin release, cAMP production and  $[\text{Ca}^{2+}]_i$  increases in isolated islets, indicating that the insulinostatic action of endogenous islet-derived ghrelin. Moreover, [D-lys<sup>3</sup>]-GHRP-6 markedly elevated GLP-1-induced insulin release, cAMP production and  $[\text{Ca}^{2+}]_i$  increases in isolated islets.

**Conclusion:** The results indicate that both exogenous and endogenous ghrelin potently attenuates glucose-dependent insulinotropic actions of GLP-1 by suppressing cAMP-mediated  $[\text{Ca}^{2+}]_i$  signaling in islet  $\beta$ -cells, while ghrelin receptor antagonist potentiates the GLP-1-induced insulin release by elevating cAMP generation and  $[\text{Ca}^{2+}]_i$  increases. These findings indicate that interaction between ghrelin and GLP-1 plays an important role in physiological regulation of glucose-induced insulin release in islet  $\beta$ -cells, and suggest that ghrelin antagonism provides a novel strategy to treat type 2 diabetes with dysregulated insulin release.

### 406

**Knockout of connexin 36 (Cx36) blocks KCl-induced exchange of ATP with the medium and glucose-induced insulin secretion in isolated mouse islets**

**J. Tamarit-Rodriguez**<sup>1</sup>, J. Pizarro-Delgado<sup>1</sup>, L.C. Barrio<sup>2</sup>, R. Martín-del-Río<sup>2</sup>, M. Romero<sup>2</sup>, D. González-Nieto<sup>2</sup>, D.L. Paul<sup>3</sup>;

<sup>1</sup>Biochemistry, Complutense University, Madrid, Spain, <sup>2</sup>Research, Hospital Ramón y Cajal, Madrid, Spain, <sup>3</sup>Neurobiology, Harvard University, Boston, USA.

**Background and aims:** Rat islets loose amino acids and adenine nucleotides after KCl-depolarization even in the absence of extracellular  $\text{Ca}^{2+}$  discarding that a mechanism of exocytosis could be implicated. The leakage of intracellular metabolites is reversible, e.g., by adding extracellular ATP, and can be partially prevented by connexin/pannexin inhibitors mefloquine, flufenamic acid. To determine whether the adenine nucleotide exchange may be mediated by hemichannels of connexin-36 (Cx36), the principal connexin expressed

in pancreas islets, we have used constitutive Cx36-deficient mice (Cx36-KO) in our studies

**Materials and methods:** Islet ATP and ADP content were measured with the luciferin/luciferase method. Insulin release was radioimmunologically determined.

**Results:** 70 mM KCl-depolarization at 5 mM glucose (G5) decreased ATP content in wild ( $1.74 \pm 0.14$ ,  $n=11$  vs  $2.55 \pm 0.23$ ,  $n=11$  pmol/islet;  $p<0.008$ ) but not in KO-islets ( $3.82 \pm 0.33$ ,  $n=8$  vs  $3.65 \pm 0.21$ ,  $n=7$  pmol/islet; N.S.). However, basal (G5) ATP content in KO-islets was much higher than in wild controls at either G5 ( $3.82 \pm 0.33$ ,  $n=7$  vs  $2.55 \pm 0.23$ ,  $n=11$  pmol/islet;  $p<0.005$ ) or G20 ( $4.82 \pm 0.56$ ,  $n=6$  vs  $3.52 \pm 0.20$ ,  $n=10$  pmol/islet;  $p<0.04$ ). G20 increased, as expected, the intracellular content in both controls ( $3.52 \pm 0.20$ ,  $n=10$  vs  $2.55 \pm 0.23$ ,  $n=11$  pmol/islet;  $p<0.005$ ) and KO-islets ( $4.82 \pm 0.56$ ,  $n=6$  vs  $3.82 \pm 0.33$ ,  $n=7$ ; N.S.). As previously observed in normal rat islets, depolarization with 70 mM KCl allowed extracellular ATP (5mM) to increase its content in islets of wild type ( $4.49 \pm 0.58$ ,  $n=7$  vs  $1.74 \pm 0.14$ ,  $n=11$  pmol/islet;  $p<0.0001$ ) but not in KO-islets ( $4.11 \pm 0.63$ ,  $n=8$  vs  $3.65 \pm 0.21$ ,  $n=7$  pmol/islet; N.S.). The ATP/ADP ratio was measured in all the experiments and it was only increased by G20 in either wild ( $2.14 \pm 0.30$ ,  $n=9$  vs  $1.14 \pm 0.15$ ,  $n=9$ ;  $p<0.004$ ) or KO-islets ( $2.29 \pm 0.37$ ,  $n=5$  vs  $1.10 \pm 0.09$ ,  $n=7$ ;  $p<0.01$ ). Perfused wild type islets showed a biphasic insulin secretory response to a change of glucose concentration from 5 to 20 mM ( $22.8 \pm 6.0$ ,  $n=5$  vs  $85.8 \pm 21.0$ ,  $n=5$  ng insulin/30 min x 40 islet;  $p<0.02$ ). By contrast, KO-islets showed a significantly lower basal (G5) release of insulin than controls ( $7.5 \pm 1.5$ ,  $n=5$  vs  $22.8 \pm 6.0$ ,  $n=5$  ng insulin/30 min x 40 islet;  $p<0.05$ ) and it was only transiently and poorly stimulated by G20 ( $19.3 \pm 4.5$ ,  $n=5$  vs  $7.5 \pm 1.5$ ,  $n=5$  ng insulin/30 min x 40 islet;  $p<0.04$ ). Extracellular ATP (5 mM) stimulated a second phase of insulin secretion in control islets depolarized with 70 mM KCl ( $42.3 \pm 7.2$ ,  $n=4$  vs  $23.0 \pm 3.9$ ,  $n=5$  ng insulin/20 min x 40 islet;  $p<0.04$ ) but not in KO-islets ( $8.7 \pm 1.7$ ,  $n=6$  vs  $6.8 \pm 1.1$ ,  $n=6$  ng insulin/20 min x 40 islet; N.S.).

**Conclusion:** i) Deficiency of Cx36 in isolated mouse islets prevented the leakage of ATP to the extracellular medium and abolished the uptake of extracellular ATP, indicating that the transmembranal flux of ATP is mediated by Cx36 hemichannels. ii) Failure of Cx36-KO islets to increase insulin secretion in response to glucose highlights the importance of both Cx36 channels and hemichannels in the  $\beta$ -cells.

Supported by: MCINN (SAF2009-1671)

## 407

### Novel 2-way communication between liver and islets mediated by follistatin, insulin and glucagon

K. Bouzakri<sup>1</sup>, P. Plomgaard<sup>2,3</sup>, C. Brandt<sup>2</sup>, B.K. Pedersen<sup>2</sup>, P.A. Halban<sup>1</sup>;

<sup>1</sup>Department of Genetic Medicine and Development, CMU, Geneva, Switzerland, <sup>2</sup>Department of Infectious Diseases, Rigshospitalet, Centre of Inflammation and Metabolism, Copenhagen, Denmark, <sup>3</sup>Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark.

**Background and aims:** Follistatin also known as activin-binding protein is a glycoprotein which binds and neutralizes several members of the TGF- $\beta$  superfamily, in particular myostatin and activin A. Recent studies have shown that plasma follistatin increases in response to exercise, or after prolonged starvation and the most likely origin is the liver. The aim was to explore the direct impact of follistatin on pancreatic islets and sorted beta-cells, as well as the impact of islet hormones on follistatin secretion.

**Materials and methods:** Primary rat beta-cells were used to explore the impact of 24 h exposure to follistatin (10 and 50 ng/mL) on apoptosis (TUNEL assay), proliferation (BrdU incorporation) and short-term insulin secretion (1h 2.8 mM glucose followed by 1h 16.7 mM glucose at the end of the 24h culture period). Human islets were exposed to 50ng/mL of follistatin for different timing up to 24h in order to explore in parallel insulin and glucagon secretion. Mice were injected intraperitoneally with glucagon (25  $\mu$ g/Kg body weight) and follistatin mRNA expression in the liver measured after 1h. Data are mean  $\pm$  SE.

**Results:** Beta-cells ( $n=4$ ) treated with follistatin for 24h showed a decrease in apoptosis when compared to untreated cells ( $0.17 \pm 0.06$  % and  $0.42 \pm 0.04$  % TUNEL positive cells for 10 and 50 ng/mL follistatin respectively vs.  $0.87 \pm 0.8$  % for control). Proliferation was increased when compared to untreated cells ( $7.73 \pm 0.37$  % and  $6.27 \pm 0.3$  % BrdU positive cells for 10 and 50 ng/mL follistatin respectively vs.  $4.0 \pm 0.17$  % for control). Interestingly, both 10 and 50 ng/mL follistatin increased basal insulin secretion respectively by  $2 \pm 0.1$  and  $2.2 \pm 0.12$ -fold, whereas glucose stimulated insulin secretion (GSIS) was decreased by  $39.2 \pm 0.56$  % (10ng/ml) and  $35.9 \pm 0.65$

% (50ng/ml) when compared to untreated cells. Human islets treated with 50ng/mL follistatin showed a decrease of basal insulin secretion ( $n=3$ ) during the first 6h of treatment and but an increase after 24h, whereas GSIS remain unchanged. Glucagon release ( $n=2$ ) at 16.7 mM glucose was decreased 4-fold after 1 and 2 h follistatin treatment but unchanged after 24h. There was no effect of follistatin on stimulated glucagon secretion (2.8 mM glucose) at any time of treatment. Mice injected with glucagon showed a 12.6-fold ( $p<0.05$ ) increase in liver follistatin mRNA 1 hour after administration compared to untreated mice.

**Conclusion:** Taken together our results show that follistatin can impact directly on beta-cell survival and proliferation as well as islet hormone secretion. Moreover, we show that glucagon can act on the liver to up regulated follistatin mRNA. These results suggest a novel 2-way axis of communication between islets and the liver, mediated by follistatin and two major islet hormones, insulin and glucagon.

## 408

### Green tea polyphenol epigallocatechin 3-gallate affects insulin secretion and AMPK in mouse and human islets through inhibition of glutamate dehydrogenase

S. Pournourmohammadi<sup>1</sup>, D. Bosco<sup>2</sup>, P. Maechler<sup>1</sup>;

<sup>1</sup>Department of Cell Physiology and Metabolism, <sup>2</sup>Department of Surgery, University of Geneva, Switzerland.

**Background and aims:** The mitochondrial enzyme glutamate dehydrogenase (GDH) participates to the control of insulin secretion and its absence in beta-cells limits the secretory response to glucose. Green tea polyphenol epigallocatechin 3-gallate (EGCG) has been shown to prevent progression to glucose intolerance in obese pre-diabetic mouse models. EGCG-mediated reduction of the secretory response could contribute to these effects and GDH is a putative target of EGCG. Here, we tested the specificity of EGCG for GDH using beta-cell specific GDH null mice ( $\beta$ Glut1<sup>-/-</sup>). Moreover, effects of EGCG on the secretory responses to different secretagogues were tested in human islets. Additionally, we investigated possible links between GDH activation and AMPK phosphorylation state.

**Materials and methods:** Mouse islets were isolated from wild type control mice (WT) and beta-cell specific GDH null mice ( $\beta$ Glut1<sup>-/-</sup>). Freshly isolated human islets were obtained from donors with the family's consent. The use of human islets for research was approved by the institutional ethic committee. The effects of 20 $\mu$ M EGCG were tested on insulin secretion in response to different insulin secretagogues (measured by RIA) and on AMPK phosphorylation state (immunoblotting).

**Results:** In WT mouse islets, EGCG reduced insulin secretion stimulated with 22.8mM glucose (-55%,  $p<0.05$ ). This inhibition was similar to the one conferred by the absence of GDH in  $\beta$ Glut1<sup>-/-</sup> islets (-52%,  $p<0.05$ ).  $\beta$ Glut1<sup>-/-</sup> islets were insensitive to EGCG as they did not exhibit further reduction of the secretory response, thereby indicating GDH-mediated action of EGCG. Addition of dimethyl-glutamate in the presence of EGCG fully restored the secretory response in WT islets. Insulin secretion stimulated by the GDH-dependent secretagogues glutamine plus BCH was markedly inhibited by EGCG (-67%,  $p<0.05$ ). In human islets, EGCG inhibited insulin secretion stimulated by 16.7mM glucose (-58%,  $p<0.05$ ) and glutamine plus BCH (-25%,  $p<0.05$ ). However, the secretory response to calcium-raising concentrations of KCl (30mM) was not changed by EGCG. Low glucose enhanced phosphorylated AMPK, an effect counteracted by incubation with GDH-dependent glutamine plus BCH, but reversed by further addition of EGCG.

**Conclusion:** The present data indicate that GDH is the main target of EGCG in mouse and human islets. The effects of EGCG on energy status demonstrated crosstalk between GDH and AMPK signalling.

Supported by: Hjelt Diabetes Foundation

## 409

### Is insulin secretion regulated by glucagon?

B. Svendsen, J. Pedersen, J.J. Holst;

The Novo Nordisk Foundation Center for Basic Metabolic Research, Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Denmark.

**Background and aims:** Glucagon is secreted from the pancreatic alpha cells in the portal blood supply in response to hypoglycemia and acts as the major counter regulatory hormone to insulin. The intra-islet states that insulin reg-



ulates glucagon by suppression, but the role of glucagon in intraislet paracrine regulation is controversial. This study aimed to unravel intraislet paracrine functions of glucagon.

**Materials and methods:** In situ perfused mouse pancreases from wildtype mice as well as glucagon receptor knockout mice (M.Channon, New York) and diphtheria toxin mediated alpha cell knock-down transgenic mice infused with low (3.5 mM) and high (15.0 mM) glucose concentrations together with arginine, glucagon, insulin and somatostatin. Effluent concentrations of insulin, glucagon and somatostatin were measured.

**Results:** Infusion of arginine increased both glucagon, insulin and somatostatin secretion in control mice from a basal insulin level of  $6.6 \pm 2.4$  pM to a peak level of  $71.6 \pm 21.4$  (p=0.0108; n=7), basal glucagon level of  $14.3 \pm 4.1$  pM to peak level of  $49.9 \pm 7.1$  (p=0.0010), and basal somatostatin level of  $7.3 \pm 1.3$  pM to  $32.3 \pm 6.4$  (p=0.0183). 15 mM glucose increased insulin secretion ( $66.3 \pm 11.6$  pM) as compared to 3.5 mM glucose ( $4.5 \pm 1.9$  pM; p=0.0001) and somatostatin (from  $7.3 \pm 1.3$  to  $29.0 \pm 8.7$  pM). Glucagon receptor knock-out mice had dramatically increased secretion of glucagon ( $AUC_{0-65 \text{ min}}$ :  $8409 \text{ pM} \cdot \text{min}$ ) compared to controls ( $1489 \pm 119.3 \text{ pM} \cdot \text{min}$ ; p<0.0001) and decreased levels of insulin. Infusion of glucagon in control mice dramatically increased insulin secretion ( $AUC_{0-65 \text{ min}}$ :  $9620 \pm 119.5 \text{ pM} \cdot \text{min}$ ) as compared to controls ( $AUC_{0-65 \text{ min}}$ :  $279 \pm 548.7 \text{ pM} \cdot \text{min}$ ; p=0.001). Diphtheria toxin destroyed 95% of the alpha cells as indicated by a reduction in pancreas glucagon content of  $57 \pm 7.8$  pmol/g compared to a normal content of 500–600 pmol/g. Furthermore, the perfused pancreas showed no glucagon response to arginine after diphtheria toxin, and reduced insulin secretion ( $AUC_{0-65 \text{ min}}$ :  $1653 \pm 193.8$ ; p=0.04; n=7) vs. controls. Glucose stimulated insulin secretion was similar in control and alpha cell knockdown mice, arginine stimulation was abolished in knock-down.

**Conclusion:** Our findings suggest that alpha cells control insulin secretion rather than the opposite. Infusion of glucagon increased insulin secretion, while insulin secretion was decreased in glucagon receptor knockout mice and mice with an alpha cell knock-down. This is in keeping with recent discoveries regarding (human) islet microvasculature, showing beta cells covered by alpha cells receiving the blood supply. In rat perfusions performed in the lab, insulin at  $10^{-7}$  M was without effect on glucagon. Arginine stimulation of insulin depends critically on paracrine glucagon.

## 410

### ICA512 luminal domain: unravelling its role in insulinoma and pancreatic beta cells

J.M. Torkko<sup>1</sup>, A. Müller<sup>1</sup>, R. Dirksen<sup>1</sup>, M.E. Primo<sup>2</sup>, L. Sosa<sup>2</sup>, A. Altkrüger<sup>1</sup>, C. Münster<sup>1</sup>, D. Richter<sup>1</sup>, A. Friedrich<sup>1</sup>, M. Sica<sup>3</sup>, M. Ermácora<sup>4</sup>, M. Solimena<sup>4</sup>;

<sup>1</sup>Molecular Diabetology, Paul Langerhans Institute Dresden, Germany, <sup>2</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (Conicet), University of Buenos Aires, Argentina, <sup>3</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (Conicet), University of Quilmes, Buenos Aires, Argentina, <sup>4</sup>Consejo Nacional de Investigaciones Científicas y Técnicas, University of Quilmes, Buenos Aires, Argentina.

**Background and aims:** ICA512/IA-2/RPTPN is a catalytically inactive receptor tyrosine phosphatase enriched in the membrane of insulin secretory granules of pancreatic beta cells. Studies in insulinoma cells indicated that calpain-mediated cleavage of its cytoplasmic tail upon granule exocytosis gives origin to a retrograde signal that allows beta cells to adjust the biogenesis of granules to their consumption. Structural studies have shown that its mature ectodomain (ME ICA512), as adhesive mucins, contains a SEA domain-like fold and displays self-proteolytic activity in vitro. Here we investigated further the properties of the ICA512 luminal/extracellular domain.

**Materials and methods:** ICA512 expression was investigated by immunoblotting and immunocytochemistry of the cells, expressing various tagged ICA512 variants and also applying the extracellular region specific antibodies in the analyses.

**Results:** We show that RESP-18 homology domain is responsible for the sorting of ICA512 into granules. Accordingly, an ICA512 mutant lacking the entire luminal domain, except for ME ICA512, is constitutively delivered to the cell surface of insulinoma INS-1 cells. Strikingly, however, this mutant is not cleaved by calpain upon glucose stimulation. Moreover, we found that the N-terminal extracellular luminal domain and the membrane integral part ME ICA512 are subject to proteolysis during the maturation of insulin secretory granules.

**Conclusion:** Hence, an intriguing scenario is that proper sorting of ICA512 into granules and processing of its luminal domain are required for calpain-induced cleavage of its intracellular region, and thus its retrograde signaling.

Supported by: DFG SFB655 research program

## 411

### Analysis of mechanisms via which pure efaroxan enantiomers increase insulin secretion from perfused mouse islets

K. Stadlbauer<sup>1</sup>, Z. Lehner<sup>1</sup>, I. Rustenbeck<sup>2</sup>, A. Fenzl<sup>1</sup>, V.A.M. de Cillia<sup>1</sup>, D. Gruber<sup>1</sup>, A. Luger<sup>1</sup>, C. Fürnsinn<sup>1</sup>;

<sup>1</sup>Dept. Med. III, Div. Endocrinol. Metab., Medical University of Vienna, Austria, <sup>2</sup>Institute of Pharmacology and Toxicology, University of Braunschweig, Germany.

**Background and aims:** Several mechanisms of action have been implicated in the direct stimulation of insulin release from pancreatic islets by imidazoline compounds, which includes antagonism at  $\alpha 2A$ -adrenoceptors, closing of KATP-channels, and others. For a long period, research efforts have focused on non-adrenergic pathways. By comparison of purified enantiomers of the imidazoline efaroxan, the present study aimed to shed more light on the relative roles of the various mechanisms.

**Materials and methods:** Isolated islets of Langerhans from C57BL mice were perfused with buffers supplemented or not with inhibitors of insulin release that oppose mechanisms putatively addressed by imidazolines: 250  $\mu$ M of the KATP-channel opener diazoxide, or 1  $\mu$ M of the  $\alpha 2$ -adrenoceptor agonist UK14,304. The effects of concomitant exposure to efaroxan enantiomers (>99% ee) or to other drugs on the rates of insulin release were determined. All data in the abstract give pg cumulative insulin released per islet during 30 min of stimulation with 20 mM glucose.

**Results:** At the employed concentrations, both inhibitors almost completely blocked insulin release (vehicle,  $206 \pm 30$ , vs 1  $\mu$ M UK14,304,  $18 \pm 6$ ; vs 250  $\mu$ M diazoxide,  $15 \pm 6$ ; p<0.002 each vs vehicle). Secretion kinetics differed between the individual  $\alpha 2A$ -antagonists, but cumulative insulin release in response to established  $\alpha 2$ -antagonists supported our basic assumption that a higher potency to counteract the  $\alpha 2A$ -agonist UK14,304 than diazoxide is associated with an  $\alpha 2A$ -antagonistic mechanism of action (25  $\mu$ M yohimbine, antagonist without imidazoline structure: under UK,  $174 \pm 26$ , vs under Dia,  $68 \pm 18$ , p<0.02; 10  $\mu$ M phentolamine, antagonist with imidazoline structure: under UK,  $286 \pm 20$ , vs under Dia,  $62 \pm 5$ , p<0.005). High concentrations of the two enantiomers of efaroxan triggered similar responses in the presence of diazoxide [(+)-efaroxan: 100  $\mu$ M,  $170 \pm 6$ , p<0.001; 250  $\mu$ M,  $500 \pm 82$ , p<0.005; (-)-efaroxan: 100  $\mu$ M,  $155 \pm 32$ , p<0.05; 250  $\mu$ M,  $576 \pm 32$ , p<0.02; p values vs. diazoxide alone; no significant difference for comparison of enantiomers]. At variance to this, inhibition of insulin release by UK14,304 was counteracted by 100-fold lower concentrations of (+)-efaroxan than (-)-efaroxan [(+)-efaroxan: 0.25  $\mu$ M,  $38 \pm 16$ , ns; 2.5  $\mu$ M,  $265 \pm 26$ , p<0.001; 25  $\mu$ M,  $576 \pm 92$ , p<0.001; (-)-efaroxan: 25  $\mu$ M,  $34 \pm 16$ , ns; 250  $\mu$ M,  $559 \pm 104$ , p<0.02; p values vs. UK14,304 alone]. The latter finding is in accordance with the previously raised contention that the  $\alpha 2A$ -antagonistic activity of efaroxan is inherent to the (+)-enantiomer, which was further supported by binding data (IC<sub>50</sub> on  $\alpha 2A$ -adrenoceptors: (+)-efaroxan: 10 nM; (-)-efaroxan: 2.6  $\mu$ M).

**Conclusion:** In contrast to previous reports describing a distinct superiority of (-)-efaroxan under diazoxide, our results suggest similar potencies of the two enantiomers in the absence of adrenergic stimulation. However, the (+)-enantiomer carries a manifold higher potency than the (-)-enantiomer to counteract inhibition of insulin release by an  $\alpha 2$ -agonist. The observed superior potency at  $\alpha 2A$ -receptors vs. KATP-channels or other targets may have been underestimated in studies done without adrenergic stimulation.

## 412

### Mathematical model of glucose and amino acid induced toxicity in pancreatic beta cells

M. Salvucci<sup>1</sup>, L. Brennan<sup>2</sup>, Z. Neufeld<sup>3,4</sup>, P. Newsholme<sup>1,5</sup>;

<sup>1</sup>School of Biomolecular and Biomedical Science, University College Dublin, Ireland, <sup>2</sup>School of Agriculture and Food Science, University College Dublin, Ireland, <sup>3</sup>Complex & Adaptive Systems Laboratory, University College Dublin, Ireland, <sup>4</sup>School of Mathematics and Physics, University of Queensland, Brisbane, Australia, <sup>5</sup>School of Biomedical Sciences, Curtin University, Perth, Australia.

**Background and aims:** Glucose and the amino acid alanine are both known to acutely stimulate insulin secretion. However, there is substantial evidence that chronic exposure to high nutrient levels promotes impairment of  $\beta$ -cells function, contributing to the development of type 2 diabetes (T2DM). The objective of this study was to combine mathematical modelling with experimental work to elucidate mechanisms of high nutrient concentration dependent toxicity.

**Materials and methods:** The effects of 24 h exposure to either high glucose (HG, 30 mmol/l) or high alanine (HA, 10 mmol/l) with respect to control (CTRL, 11.1 mmol/l glucose) were investigated using a clonal rat insulin secreting cell line (BRIN-BD11). An ordinary differential equations based simplified kinetic model of core mitochondrial metabolic processes leading to ATP production (TCA cycle, alanine-specific reactions, respiratory chain, ATPase and proton leak) was built and numerically simulated in MATLAB.

**Results:** Both HG and HA chronic exposure resulted in no significant loss of cellular viability respect to CTRL. However, both treatments considerably reduced chronic insulin secretion from a control value of  $40.9 \pm 12.3$  ng/mg protein/24 h to  $35.8 \pm 9.7$  ng/mg protein/24 h for HG and  $27.3 \pm 8.3$  ( $P < 0.01$ ) for HA. Acute stimulation (20 min) with 16.7 mmol/l glucose plus 10 mmol/l alanine following HG or HA incubation also resulted in a significant reduction of insulin secretion compared with CTRL by 22 % ( $P < 0.05$ ) and 46 % ( $P < 0.01$ ) respectively. However, acute insulin secretion induced by 10 mmol/l alanine following HG culture and by 16.7 mmol/l glucose following HA exposure were found unaffected, suggesting that a selective desensitization. Moreover, KCl-induced insulin secretion was unaffected by either treatment. 24 h exposure to either HG or HA led to a marked decrease in ATP production from a CTRL value of  $2.67 \pm 0.91$  nmol/mg protein/24 h to  $2.32 \pm 1.33$  nmol/mg protein/24 h and  $1.12 \pm 0.15$  nmol/mg protein/24 h ( $P < 0.01$ ), respectively. Mathematical modelling was employed to identify key metabolic reactions whose alteration could minimize ATP production. Simulations indicated the enzymatic activities of lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH), alanine amino-transferase (ALT) and aspartate amino-transferase (AST) as potential candidates. Following long term exposure to either HG or HA, LDH activity exhibited a 2.34 fold increase ( $P < 0.001$ ) and 1.59 fold increase ( $P < 0.01$ ), respectively. However, prolonged culture in HG or HA diminished AST activity by 57.33 % ( $P < 0.01$ ) and 35.93 % ( $P < 0.01$ ), respectively. ALT activity was not significantly altered while GDH activity was significantly decreased by 36.17 % ( $P < 0.01$ ) following 24 h HA incubation.

**Conclusion:** Experimental data and model simulations combined suggest that high nutrient concentrations exert a detrimental effect on  $\beta$ -cell insulin secretion via mitochondrial metabolism adaptations. Simulations suggest that pyruvate dehydrogenase and pyruvate carboxylase activities may be markedly altered following HG or HA chronic exposure. Ongoing experimental work is being carried out to confirm predictions.

*Supported by: IRCSET*

## 413

### $\text{Ca}^{2+}$ -dependent desensitisation of insulin secretion by strong potassium depolarisation

M. Belz, K. Hatlapatka, A. Paufler, K. Schumacher, M. Willenborg, I. Rustenbeck;  
University of Braunschweig, Germany.

**Background and aims:** Depolarization by a high  $\text{K}^+$  concentration is a widely used experimental tool to stimulate insulin secretion. However, prolonged exposure to high  $\text{K}^+$  concentrations may induce counterregulatory effects which lead to the inhibition of secretion. Here the effects occurring after the initial rise in secretion were investigated.

**Methods:** Insulin secretion was measured by perfusion of NMRI mouse islets and ELISA of the fractionated efflux. The free cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) was measured by the Fura-technique, the plasma membrane potential by a standard patch-clamp technique using the perforated-patch configuration.

**Results:** After the initial peak a fast decline occurred followed by a slowly progressive decrease of secretion, which was the stronger the higher the initial peak was. At 40 mM KCl, but not at lower concentrations the decrease continued when the glucose concentration was raised from 5 to 10 mM, suggesting an inhibitory effect of the  $\text{K}^+$  depolarization. At the end of the glucose perfusion (min 120), e.g. the secretion rate in the presence of 22.5 mM  $\text{K}^+$  was significantly higher than in the presence of 40 mM  $\text{K}^+$ . When tolbutamide was added instead of raising the glucose concentration a complete inhibition down to prestimulatory values was observed within 60 min from the beginning of the depolarization by 40 mM  $\text{K}^+$ . Equimolar reduction of the NaCl concentration to preserve isoosmolarity enabled an increase of secretion in response to glucose. Unexpectedly, the same was true when the  $\text{Na}^+$ -reduced media were made hyperosmolar by choline chloride or mannitol. The insulinotropic effect of tolbutamide was not rescued by the compensatory reduction of NaCl. Since the effect of 10 mM glucose and tolbutamide on membrane potential and  $[\text{Ca}^{2+}]_i$  were both intact, this suggests a requirement

for activated energy metabolism to permit the rescue by the decreased NaCl concentration. The progressive inhibitory effects of  $\text{K}^+$  depolarization could not be explained by a loss of depolarizing strength or by a diminished  $[\text{Ca}^{2+}]_i$ . However the elevated chloride concentration of the medium could play a role in the inhibition by 40 mM KCl. Thus we created a depolarizing medium with 40 mM  $\text{K}^+$  but with an unchanged  $\text{Cl}^-$  concentration by using potassium citrate and potassium hydroxide which resulted in an attenuated inhibition of secretion. Interestingly under this condition the steady state  $[\text{Ca}^{2+}]_i$  elevation was much lower than the one induced by the use of 40 mM KCl, indicating a chelate complexation of  $\text{Ca}^{2+}$ . When we used EGTA as substitute of citrate as  $\text{Ca}^{2+}$  buffer and 40 mM of KCl, the same phenomenon could be observed.

**Conclusion:** These data suggest that a strong, but not a moderate depolarisation by  $\text{K}^+$  induces a  $[\text{Ca}^{2+}]_i$ -dependent progressive desensitization of the secretory machinery. In contrast, the decline immediately following the initial peak of secretion may result from the inactivation of voltage-dependent  $\text{Ca}^{2+}$  channels.

*Supported by: DDG, DDS, DFG*

## 414

### Pancreatic role for GLUT2 in human neonates evaluated by new variants identified in patients suffering from congenital hyperinsulinism

M. Le Gall<sup>1</sup>, A. Michau<sup>1</sup>, M. Keck<sup>1</sup>, S. Lhoste<sup>1</sup>, T. Grand<sup>1</sup>, A. Grosfeld<sup>1</sup>, P. Serradas<sup>1</sup>, J. Teulon<sup>1</sup>, P. De Lonlay<sup>2</sup>, E. Brot-Laroche<sup>1</sup>, A. Leturque<sup>1</sup>;  
<sup>1</sup>Inserm UMRS872, Paris, <sup>2</sup>Hôpital Necker Enfants Malades, Paris, France.

**Background and aims:** GLUT2 is a facilitative sugar transporter with low affinity and high capacity allowing large fluxes of sugar at physiological concentrations. GLUT2 is also a glucose receptor, detecting extracellular sugar and transducing a signal independent of glucose metabolism. The implication of GLUT2 in insulin secretion by pancreatic beta cells remains to be clarified in human although GLUT2 expression in beta cells was reported in neonates. Mutations in GLUT2 are associated with Fanconi-Bickel syndrome (FBS); patients suffer from glycogenosis and glucose homeostasis disorders improving with age. Neonates suffering from Congenital Hyperinsulinism syndrome (CHI) show severe hypoglycemia due to high insulin secretion. Mutations in genes coding for the pancreatic ATP-sensitive  $\text{K}^+$  channel are responsible for 50% of CHI, thus mutations in other genes are explored. Our hypothesis is that constitutive activation of human GLUT2 functions could be consistent with CHI syndrome. The aim of this study was thus to evaluate the role of GLUT2 in human neonate pancreatic function investigating impact of hGLUT2 natural variants on insulin secretion.

**Materials and methods:** We generated, by site-directed mutagenesis, a panel of 9 hGLUT2 natural variants to test their biological activity. We produced 4 single-point mutations suspected to abolish sugar transport, identified in FBS patients, and 3 single-point mutations as putative activating mutations identified in CHI patients. 2 single nucleotide polymorphisms (SNP) associated with diabetes or sugar-preference were also analyzed. Expression and cellular location of corresponding GLUT2 proteins were evaluated by western-blot and immunofluorescence. GLUT2-transporter kinetic parameters were calculated by measuring the uptake of radio-labeled 2-deoxy-D-glucose (2DOG) in *Xenopus* oocytes. GLUT2-receptor function was assessed by measuring glucose-sensitive gene mRNA accumulation in mhAT3F hepatoma cells. Impact on insulin secretion was assayed in insulin-secreting Min6 cells.

**Results:** The SNPs P68L and T110I did not modify GLUT2 transport activity, whereas the 4 FBS-associated mutations conducted to loss of GLUT2 function, due to altered location for G20D and S242R or lack of transport activity for P417L and W444R. Conversely, CHI-associated GLUT2 variants transported 2DOG and displayed modest alterations of kinetic parameters. Interestingly, G20S and L368P CHI variants stimulated insulin secretion by Min6 cells even in absence of glucose. This disqualified increased sugar transport as the single signaling event, and suggested that activation of receptor function of hGLUT2 may be in part responsible for the increased insulin secretion. Accordingly, L368P CHI variant displayed constitutive activation of GLUT2 receptor function since glucose-sensitive gene mRNA remained constantly high. In a pancreas biopsy of CHI a neonate, hGLUT2 was detected in insulin positive cells.

**Conclusion:** With this work, we involve human GLUT2 as an actor in insulin secretion process not only through its transporter- but also through its receptor-function in neonates. Activating mutations of GLUT2 can increase insulin secretion even in absence of glucose, we propose GLUT2 as a candidate gene to be sequenced in CHI patients.

*Supported by: INSERM, CNRS, UPMC*

## PS 018 Islet cell development and generation I

415

### The conditional inactivation of Arx in pancreatic alpha cells

M. Courtney<sup>1,2</sup>, E. Gjernes<sup>1,2</sup>, C. Ravaud<sup>1,2</sup>, N. Druelle<sup>1,2</sup>, B. Faurite<sup>1,2</sup>, P. Collombat<sup>1,2</sup>;

<sup>1</sup>Université de Nice-Sophia Antipolis, Nice, <sup>2</sup>Inserm U1091, Diabetes Genetics Team, Nice, France.

**Background and aims:** The understanding and elucidation of the genetic determinants underlying pancreatic development may potentially aid the design of cell replacement therapies to treat type 1 diabetes mellitus. In this context, we have previously shown that endocrine fate specification largely rests on the interplay between two transcription factors, Pax4 and Arx. Indeed, if Pax4 predominates, the beta- and delta-cell lineages will be specified, whereas Arx will favour alpha-cell commitment. In addition, Arx and Pax4 were found to mutually inhibit each other's transcription through direct physical interaction with the pertinent promoter. We have previously demonstrated that the ectopic expression of Pax4 in pancreatic alpha-cells results in oversized islets primarily composed of functional insulin-expressing cells displaying most characteristics of true beta-cells. In addition, lineage-tracing experiments demonstrated a regeneration and conversion of glucagon-positive cells into insulin-expressing cells. Taking into account these data and the previous finding that Arx and Pax4 are mutually inhibitory, we sought to investigate whether the ectopic expression of Pax4 in glucagon-expressing cells and the ensuing alpha-to-beta cell conversion is a direct consequence of Pax4 expression or whether the selective inhibition of Arx expression in these glucagon-expressing cells is sufficient to induce such conversion.

**Methods and results:** Taking advantage of the Cre-LoxP system, we generated transgenic mice able to conditionally inactivate the expression of Arx in glucagon-expressing cells. These mice appear healthy and display normal basal glycaemic levels. Immunohistochemical analyses of double-transgenic mice revealed hypertrophic islets associated with a hyperplasia of insulin-expressing cells (up to 8 times more insulin-positive cells compared to their wild-type counterparts). Further characterisation revealed that these insulin-expressing cells displayed many of the classical  $\beta$ -cell markers and were functional when challenged with large doses of glucose. The introduction of ROSA26- $\beta$ -Gal to our system will allow us to perform lineage-tracing experiments to help us ascertain the origin of these beta-like cells. These results will be presented.

**Conclusion:** Our preliminary findings suggest that the conditional inactivation of the transcription factor Arx in glucagon-expressing cells results in hyperplastic islets filled with  $\beta$ -like cells. Should the inhibition of Arx activities be sufficient to induce the conversion of glucagon-producing cells into beta-like cell, this factor would represent a target of choice for therapies aiming at regenerating beta-cells in vivo.

Supported by: JDRF

416

### Role of aldehyde dehydrogenase 1 (ALDH1) activity in the developing human pancreas

J. Li, M. Wong, W. Rajan, Z.C. Feng, R. Wang;

Physiology & Pharmacology, Children's Research Institute, the University of Western Ontario, London, Canada.

**Background and aims:** Understanding the nature of islet progenitor cells and the signals that regulate neogenic activity is essential to precede any application of stem cell and islet biology for cell replacement therapies for the treatment of diabetes. ALDH1, a marker for normal and malignant human stem cells, regulates the conversion of retinaldehydes to retinoic acids (RA). However, data on the expression and functional role of ALDH1 in the developing human pancreas is limited. The aim of the present study is to characterize the ALDH1 expression patterns and functional role during islet cell differentiation in the developing human pancreas.

**Materials and methods:** Expression of ALDH1 and downstream signaling molecules in the developing human (8–21 week fetal age) pancreas were examined using microarray, quantitative RT-PCR, western blotting, immunohistological and cell sorting approaches. Human fetal islet epithelial cell clusters isolated from 15–18 weeks were cultured and treated with ALDH1

inhibitor, diethylaminobenzaldehyde (DEAB) and/or retinoic acid receptor agonists (e.g. all-trans-retinoic acid [ATRA]) for up to 5 days.

**Results:** A significant change in ALDH1 expression and ALDH-regulated RA signaling molecules was observed in the developing human pancreas. ALDH1 expression was localized to ductal cells and newly formed endocrine cells (glucagon+ and insulin+), and was frequently co-localized with key transcription factors (e.g. PDX1, SOX9 and NGN3) as well as stem cell surface markers (e.g. c-Kit) during islet cell differentiation. A significant increase ALDH1 expression in SOX9+ and NGN3+ cells was observed at 20–21 week of fetal age ( $p < 0.05$ ), however, a high level of ALDH1 colocalized with insulin observed early in development was significantly reduced as development progressed ( $p < 0.05$ ). Inhibition of ALDH1 using DEAB resulted in reduced endocrine cell differentiation together with a significant increase in cell apoptosis ( $p < 0.01$ ). Furthermore, these effects of DEAB on human islet-epithelial cell differentiation could be reversed by the co-treatment of the ATRA. In addition, the ATRA effect on islet differentiation was dose-dependent, suggesting that the ability of ALDH1 to generate retinoic acids is important in regulating islet cell differentiation.

**Conclusion:** Our findings indicate that ALDH1+ cells could represent precursor cells in the developing human pancreas and inhibition of its activity and retinoid signaling could impart human fetal islet cell differentiation and survival.

Supported by: Canadian Diabetes Association

417

### Potential involvement of interleukin-6 in pancreatic alpha cell ontogeny

J. De Toro-Martín<sup>1</sup>, E. Fernández-Millán<sup>1,2</sup>, E. Lizárraga-Mollinedo<sup>1,2</sup>, F. Escrivá<sup>1,2</sup>, C. Álvarez<sup>1,2</sup>;

<sup>1</sup>Bioq. y Biol. Mol. II, Facultad de Farmacia, UCM, Madrid, <sup>2</sup>Ciberdem, ISCIII, Madrid, Spain.

**Background and aims:** The control of glucose homeostasis by the islets of Langerhans depends mainly on the coordinated secretion of glucagon and insulin by alpha- and beta-cells, respectively. The circulating levels of these hormones are partly determined by the number of these two endocrine cells types. During the early postnatal period, plasma glucagon concentration raises acutely. This condition is associated with an increase in alpha-cell mass but the trophic factors that regulate alpha-cell function and turnover during perinatal period have not been studied. Recently, pancreatic alpha-cells have been identified as a primary target of IL-6 actions regulating alpha-cell mass expansion and promoting glucagon expression and release. On the other hand, macrophage infiltrations are present in neonatal pancreas. All these premises have prompted us to investigate the physiological relevance of IL-6 to normal pancreatic alpha-cell development.

**Materials and methods:** The experiments were carried out in Wistar rats from fetal to adult age. Circulating glucagon and IL-6 were measured by RIA and ELISA, respectively. Histochemical and morphometric analyses were performed to quantify alpha-cell fraction, replication and apoptosis as well as the presence of CD68+ cells. Pancreatic IL-6 production was determined by RT-qPCR and immunostaining. To address whether IL-6 is affected in an animal model characterized by altered alpha-cell mass we used an under-nutrition rat model achieved by restricting maternal nutrition from the last third of gestation onwards to 35% of *ad libitum* intake.

**Results:** Alpha-cell fraction increased sharply after birth peaking on postnatal day 4 (PN4) and progressively decreasing to adult age. Similarly, pancreatic alpha-cell proliferation was as high as 3.5% at the end of gestation and 2.5% at the beginning of lactation but it diminished with time until values of 1.2% in adults. On the other hand alpha-cell apoptosis was low along the neonatal period but increased significantly at adult age. Serum glucagon levels paralleled the changes observed in the number of alpha-cells during development. However, circulating levels of IL-6 did not change with time suggesting no systemic effect on pancreas. In order to study the local production of IL-6, the mRNA expression of IL-6 in pancreas was analyzed. By PN4 IL-6 mRNA increased a 50% as compared to fetal age, then on PN14 the expression of IL-6 return to fetal values and finally, it decreased below fetal levels at the end of suckling time. Immunostaining of pancreatic sections localized the IL-6 within the islets mainly to the alpha-cells with no association to CD68+ cells. Undernourished (UN) rats showed decreased alpha-cell mass but normal islet architecture. Pancreas IL-6 mRNA expression was also reduced in UN rats and immunostaining of IL-6 showed delayed appearance of this cytokine within islets as compared to controls.

**Conclusion:** Birth represents an abrupt change from a high-carbohydrate to a high-fat and low-carbohydrate diet. This condition is associated with an



increment in alpha-cell mass. We found herein a transitory islet-derived IL-6 to the neonatal rat pancreatic alpha-cell, which may be required for ensuring alpha-cell mass expansion during this period.

Supported by: MINECO (BFU 2011-25420), CIBERDEM (ISCIII), Spain

## 418

### Non-invasive monitoring of pancreas transduction and beta cell mass in vivo with viral vectors

M. Morro<sup>1,2</sup>, R. Lage<sup>1,2</sup>, A. Casellas<sup>1,2</sup>, V. Jimenez<sup>1,2</sup>, C. Mallol<sup>1,2</sup>, S. Marletta<sup>1</sup>, J. Teichenne<sup>1</sup>, X. Leon<sup>1,2</sup>, M. Molas<sup>1,2</sup>, F. Kreppel<sup>3</sup>, S. Kochanek<sup>3</sup>, F. Bosch<sup>1,2</sup>, E. Ayuso<sup>1,2</sup>;

<sup>1</sup>Center of Animal Biotechnology and Gene Therapy and Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Bellaterra, Spain, <sup>2</sup>CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain, <sup>3</sup>Department of Gene Therapy, University of Ulm, Germany.

**Background and aims:** The determination of  $\beta$ -cell mass in living mice is an important limitation in assessing the efficacy of those diabetes therapies aimed at protecting or regenerating insulin-producing cells. A non-invasive method that allowed the measurement, with high accuracy, of the  $\beta$ -cell mass over a period of time would greatly benefit diabetes research. Secretable alkaline phosphatase (seAP) is an attractive gene marker for non-invasive monitoring since it is secreted to the bloodstream, it is not immunogenic in mice, and detection by luminometric methods is highly sensitive. Serum values of seAP are directly correlated to  $\beta$ -cell mass when this gene is expressed under the control of Pdx-1 promoter in transgenic mice. We and others have shown that exocrine and endocrine pancreas can be genetically manipulated with adeno-associated and adenoviral vectors. Here, we aimed to use viral vectors expressing seAP to monitor pancreas transduction over time and to correlate seAP levels with  $\beta$ -cell mass.

**Materials and methods:** We have generated AAV9 and helper-dependent adenoviral (HD-Ad) vectors expressing the secretable Alkaline Phosphatase (seAP) under the control of the insulin (ins) and elastase (ela) promoter. These vectors were injected intraductally to healthy or diabetic mice.

**Results:** We demonstrate that intraductal delivery of AAV9-Ins-seAP and AAV9-ela-seAP vectors resulted in long term (>6 months) detection of seAP in the serum of healthy mice. Importantly, when AAV9-Ins-seAP vectors were administered to diabetic mice, seAP levels were almost undetectable, correlating with the reduced  $\beta$ -cell mass of these animals. In addition to AAV, HD-Ad are also promising tools for gene transfer since they can accommodate large transgenes and mediate long-term expression in animal models. For the first time, we demonstrate here that intraductal delivery of HD-Ad (Ins-seAP) vectors to healthy mice resulted in persistent (>5 months) circulating levels of seAP, indicating long-term transduction of  $\beta$ -cells.

**Conclusion:** We have shown that seAP is a useful transgene for non-invasive monitoring of pancreas transduction and viral vectors expressing seAP could be used to monitor changes in  $\beta$ -cell mass in mice

Supported by: EFSF/JDRF/Roche: Young Investigator Award in Innovative Therapy for T1D

## 419

### Highly efficient pancreatic mouse islets due to activating glucokinase gene mutations: lessons from human models

F. Martin<sup>1</sup>, M. Navarro<sup>1</sup>, R. Araujo<sup>1</sup>, C. Vazquez<sup>2</sup>, E. Baixeras<sup>2</sup>, M. Repice<sup>2</sup>, A. Izarra<sup>3</sup>, N. Rodriguez<sup>2</sup>, J. Dominguez-Bendala<sup>4</sup>, A. Bernad<sup>3</sup>, A. Cuesta<sup>2</sup>;

<sup>1</sup>CABIMER-CIBERDEM, Seville, Spain, <sup>2</sup>Fundacion IMABIS, Malaga, Spain, <sup>3</sup>CNIC, Madrid, Spain, <sup>4</sup>Diabetes Research Institute, Miami, USA.

**Background and aims:** Humans affected with severe neonatal hypoglycemia due to GCK activating mutation (GCK-Hypoglycemia) present structurally well formed and very large islets,  $\beta$ -cell proliferation and some degree of apoptosis. In an affected patient reported, after a 98% pancreatectomy, the approximately 20,000 islets left, allowed the patient to live a normal life without developing diabetes. This finding is the proof of concept that a supra-physiological activation of glucokinase leads, in humans, to what should be considered, “highly efficient pancreatic human islets”. This finding opens a new and fascinating door to explore novel approaches to cell therapy as a treatment of diabetes. Our aim is to “in vitro” replicate these phenomena as a novel approach to diabetes cell therapy.

**Materials and methods:** Mouse islets were infected using lentiviral technology with the empty vector (pRRL.cmv.ires.eGFP) (eGFP islets), the vector containing wild type GCK (pRRL.cmv.GCK-WT.ires.eGFP) (GCK-WT islets) and the activating GCK mutation V91L found in the patient (pRRL.cmv.GCK-V91L.ires.eGFP) (GCK-V91L islets). After 6 and 15 days of infection, GSIR at different glucose concentrations (1.1, 2.8, 4.2, 5.6, and 8.3 mmol/l),  $\beta$ -cell proliferation (BrdU incorporation and Ki67 expression) and apoptosis (TUNEL imaging assay) was studied.

**Results:** The infection efficiency was 93% (n= 8 infections). GSIR (6d after infection) was significantly higher ( $p<0.05$ ; n= 5 infections, 15 GSIR experiments) above 4.2 mmol/l glucose in GCK-V91L islets, as compared to the rest of groups. Moreover, GCK-V91L islets started GSIR at lower glucose concentrations (4.2 mmol/l). After 15d of infection, GSIR was significantly higher ( $p<0.01$ ; n= 3 infections, 12 GSIR experiments) at all glucose concentrations in GCK-V91L islets. BrdU incorporation (15d after infection) was 6.8 fold higher (n= 3 infections, 60 islets analyzed) in GCK-V91L islets than in non-infected control and eGFP islets, and 5.1 fold higher (n= 3 infections, 60 islets analyzed) when compared with GCK-WT islets. Ki67 positive cells (15d after infection) were significantly higher ( $p<0.05$ ; n= 2 infections, 60 islets analyzed) in GCK-V91L islets as compared with the rest of groups. Apoptosis (15 d after infection) was significantly higher ( $p<0.05$ ; n= 2 infections, 28 islets analyzed) in GCK-V91L islets versus rest of groups.

**Conclusion:** We have been able to replicate “in vitro” the highly efficient islets described in the patient with severe neonatal GCK-Hypoglycemia. These islets could be used as a new tool, for diabetes cell therapy, to overcome the shortage of islets due to insufficient donors and problems in islet isolation.

Supported by: SAF 2010-22113, PI0608-2010, PI0022-2008, CTS-6747, BIO311

## 420

### Accelerating the in vivo maturation of macro-encapsulated human embryonic stem cell derived-pancreatic progenitor cells by manipulating NKX6.1 expression in culture

J.E. Bruin<sup>1</sup>, J.K. Fox<sup>1</sup>, K. Narayan<sup>2</sup>, J. Xu<sup>2</sup>, A. Rezanian<sup>2</sup>, T.J. Kieffer<sup>1</sup>;

<sup>1</sup>Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada, <sup>2</sup>BetaLogics Venture, Janssen Research & Development, Raritan, USA.

**Background and aims:** Human embryonic stem cells (hESCs) are considered a potential alternative to cadaveric islets as a source of transplantable cells for treating patients with diabetes. We previously described a 4-stage, 14-day protocol that efficiently differentiated hESCs *in vitro* into a highly enriched pancreatic progenitor cell population, composed of mainly pancreatic endoderm cells (PDX1/NKX6.1 co-positive) and polyhormonal cells (insulin/glucagon co-positive). Following a lengthy maturation period (~30 weeks) in diabetic mice, these cells secreted human insulin in a meal- and glucose-dependent manner and were capable of treating pre-existing hyperglycemia. In the current study, we aimed to understand the respective roles of pancreatic endoderm and polyhormonal cells for *in vivo* maturation by manipulating the NKX6.1-expressing cell population in culture.

**Materials and methods:** Three different populations of pancreatic progenitor cells (expressing high, medium or low levels of NKX6.1) were generated *in vitro* with modified versions of our previously reported 14-day differentiation protocol and characterized by flow cytometry pre-transplant. Progenitor cells were transplanted subcutaneously within Theracyte<sup>TM</sup> encapsulation devices (5x10<sup>6</sup> cells/device) into SCID-beige mice with STZ-induced diabetes (n=8/group). Maturation of encapsulated cells was assessed by blood glucose tracking and meal/arginine challenges throughout the 5-month study.

**Results:** Prior to transplant all hESC-derived cell populations contained a high proportion of PDX1-positive cells (~85-90%), but were distinguished by their high (~80%), medium (~60%) or low (~25%) expression of NKX6.1. This was inversely proportional to the endocrine population (~13, 20 and 60% synaptophysin-positive cells in NKX6.1-high, -med and -low cultures, respectively). Endocrine cells in all groups were largely polyhormonal (insulin/glucagon co-positive) prior to transplant. Mice transplanted with NKX6.1-low cells remained hyperglycemic throughout the 5-month post-transplant period relative to the NKX6.1-med and -high groups. Overnight fasting human C-peptide levels were similar in all groups throughout the study, but the NKX6.1-high group displayed a significant increase in human C-peptide secretion following a meal challenge at 3 and 4 months post-transplant. Similarly, at 17 weeks arginine-stimulated human insulin secretion was only observed in the NKX6.1-high group; fasting glucagon levels were significantly higher in the NKX6.1-low group during this challenge.

**Conclusion:** hESC-derived cells containing relatively high proportions of NKX6.1-positive and low endocrine-positive cells matured more quickly into insulin-secreting cells with improved function compared to NKX6.1-med or -low cell populations *in vivo*. This accelerated maturation period may be beneficial for translating a progenitor cell-based therapy for clinical transplantation in patients with diabetes.

*Supported by:* CIHR, SCN, JDRF

## 421

### Induction of gastrointestinal hormones in pancreatic islets of a mouse model of beta cell regeneration

K. Minami<sup>1</sup>, H. Kitanoya<sup>1</sup>, H. Takahashi<sup>1</sup>, S. Seino<sup>1,2</sup>;

<sup>1</sup>Division of Cellular and Molecular Medicine, <sup>2</sup>Division of Diabetes and Endocrinology, Kobe University Graduate School of Medicine, Japan.

**Background and aims:** We previously reported that pancreatic beta cells are spontaneously regenerated in mice expressing a dominant negative form of Kir6.2 gene specifically in the beta cells (Kir6.2G132S Tg mice). Although Tg mice exhibit severe hyperglycemia and marked loss of pancreatic beta cells at 4 weeks of age, the hyperglycemia is improved by concomitant improvement of pancreatic insulin content and beta cell mass at 25 weeks of age or older. However, the mechanism of regeneration of pancreatic beta cells in Kir6.2G132S Tg mice has remained unclear. We have addressed this issue by using comparative transcriptome analysis between pancreatic islets of Kir6.2G132S Tg mice and those of wild-type mice.

**Materials and methods:** Kir6.2G132S Tg mice (line M45) were originally generated in BDF1 mice and backcrossed to C57BL/6J mice. Changes in body weights, blood glucose levels, and serum insulin levels were measured. Pancreatic islets were obtained from mice at 4 (hyperglycemic state) and 25 weeks of age (improved glycemic state) by collagenase digestion and were subjected to GeneChip<sup>TM</sup> transcriptome analysis (n=3). Differential expression of genes was confirmed by quantitative real-time RT-PCR analysis. For immunohistochemistry, mice were perfused with phosphate-buffered paraformaldehyde to fix the tissues. Sections were prepared from the fixed tissues and subjected to immunostaining.

**Results:** At 4 weeks of age, the number of insulin-positive cells was reduced significantly in Kir6.2G132S Tg mice compared to wild-type mice. However, the number gradually increased with age: the Tg pancreas at 25 weeks of age was histologically indistinguishable from wild-type pancreas. We found that 255 and 309 genes were up-regulated (> 5-fold) in Kir6.2G132S Tg islets at 4 and 25 weeks of age, respectively. Expressions of 81 genes were increased at both 4 and 25 weeks of age. These include growth factors, gastrointestinal hormones, and transcription factors. In addition, 123 and 228 genes were down-regulated (> 5-fold) in Kir6.2G132S Tg islets at 4 and 25 weeks of age, respectively; 21 genes were decreased at both 4 and 25 weeks of age. Interestingly, more than 100-fold increase in gastrin gene expression was found in Kir6.2G132S Tg islets at 4 weeks of age. Indeed, immunoreactivity of gastrin was detected in the Tg islets. Up-regulation of gastrin gene was also evident at 25 weeks of age but the difference became small (9-fold). Furthermore, gene expression of another gastrointestinal hormone cholecystokinin (CCK) was increased in Kir6.2G132S Tg islets compared to wild-type islets (9-fold and 51-fold increase at 4 and 25 weeks, respectively). There was no significant difference in either CCK receptor (CCKAR) or gastrin receptor (CCKBR) expression between wild-type and the Tg islets as judged by GeneChip<sup>TM</sup> analysis.

**Conclusion:** Gastrin and CCK were up-regulated in Kir6.2G132S Tg islets in the course of recovery of glycemia and beta cell mass. Autocrine/paracrine signals of these gastrointestinal hormones may have roles in the regeneration of pancreatic beta cells in Tg mice.

*Supported by:* CREST; MEXT

## 422

### Fibrin-integrin support of beta cell differentiation, function and proliferation

M. Riopel<sup>1,2</sup>, J. Li<sup>1,3</sup>, W. Stuart<sup>1,2</sup>, R. Wang<sup>1,3</sup>;

<sup>1</sup>Children's Health Research Institute, <sup>2</sup>Pathology, Western University,

<sup>3</sup>Physiology and Pharmacology, Western University, London, Canada.

**Background and aims:** Extracellular matrix (ECM)-integrin stimulation has been shown to promote beta cell differentiation, function and proliferation. However, islets cultured on ECM proteins do not maintain islet archi-

tecture or long-term insulin expression and cannot form 3D scaffolds for *in vivo* transplantation. Fibrin is a provisional matrix protein that interacts with Arg-Gly-Asp-binding receptors, including  $\alpha 5\beta 1$  and  $\alpha V\beta$  integrins. It is used routinely as a biomaterial scaffold in surgery and tissue engineering, and was reported to support human and rat adult islet function. However, the role of fibrin-integrin interactions in support of beta cells has not been investigated. The objective of the current study is to assess the inter-relation between fibrin and integrins in promoting beta cell differentiation, function and proliferation.

**Materials and methods:** Isolated human fetal islet-epithelial cell clusters (18–21 weeks of age) and INS-1 cells were cultured with fibrin in 2D and 3D, and on tissue culture polystyrene (TCPS) for 1–4 weeks then analyzed by quantitative RT-PCR, western blot, immunofluorescence and ELISA techniques.

**Results:** The mRNA expression integrins  $\alpha V$  and  $\beta 3$  was significantly up-regulated along with PDX1, INS and GCG in human fetal islet-epithelial cells cultured with fibrin when compared to TCPS culture. This was associated with increased protein level of PDX1,  $\alpha V$  integrin and  $\beta 3$  integrin. Alongside, INS-1 cells cultured with fibrin showed increased mRNA expression of integrin receptors  $\beta 1$ ,  $\beta 3$  and  $\alpha V$ , with significantly increased expression of the *Ins1* gene ( $p < 0.001$ ) compared to control. Fibrin-culture of INS-1 cells also showed islet-like cluster formation. In addition, basal insulin secretion was significantly increased in INS-1 cells cultured on 2D fibrin ( $p < 0.01$ ), while glucose-stimulated insulin secretion was significantly higher in 3D fibrin culture ( $p < 0.05$ ) compared to controls. Also, fibrin promoted cell proliferation in human fetal islet-epithelial cell clusters and INS-1 cells after 1 week culture compared to TCPS culture ( $p < 0.01$ ). Furthermore, fibrin-cultured INS-1 cells showed preserved Pdx1 expression in nuclei with a significant decrease in apoptosis ( $p < 0.05$ ).

**Conclusion:** Fibrin culture of human fetal islet-epithelial cell clusters and INS-1 cells showed significantly increased cell differentiation, function and proliferation with decreased cell apoptosis. These improvements were corroborated with significantly increased  $\alpha V$ ,  $\beta 1$  and  $\beta 3$  integrin receptor expression. These results suggest that fibrin's ability to promote islet cell maintenance depends on up-regulation of key integrin receptors. Integrin blocking experiments will be conducted to understand the mechanisms behind fibrin's effects. Culturing pancreatic cells with fibrin may be beneficial in order to expand beta cell mass and differentiate precursors to beta cells to generate a cell source for use in cell-based therapies.

*Supported by:* NSERC

## 423

### Predominance of beta cell neogenesis in human at early stage of glucose intolerance

S. Yoneda, S. Uno, H. Iwahashi, A. Yoshikawa, J. Kozawa, K. Okita,

A. Imagawa, I. Shimomura;

Metabolic Medicine, Graduate School of Medicine, Osaka University, Japan.

**Background and aims:** Beta cell mass is regulated by a balance of beta cell neogenesis, replication and apoptosis. Recent studies have showed that beta cell mass decreased, beta-cell apoptosis increased and beta-cell neogenesis occurred in type 2 diabetes. However, it is unclear how beta-cell mass and the incidence of beta-cell neogenesis, replication and apoptosis change at each glucose tolerance stage in type 2 diabetes, especially in prediabetes.

**Materials and methods:** We classified 26 patients with a 75 g oral glucose tolerance test and their past history into 9 normal glucose tolerance (NGT), 9 impaired glucose tolerance (IGT), 8 newly-diagnosed diabetes mellitus (NDM) and 7 longstanding type 2 diabetic patients (LDM) before partial pancreatectomy, and quantitatively evaluated pancreatic tissues from those patients by immunohistochemical approach. The mean age of these patients composed of 13 males and 20 females was 68 years old, 17 patients had been diagnosed with pancreatic cancer, 4 with bile duct cancer, 9 with intraductal papillary mucinous neoplasm, 1 with carcinoma of the papilla of Vater, 1 with serous cyst adenoma, 1 with well-differentiated endocrine cancer. The mean diabetes duration of LDM group was 18.8 years.

**Results:** The relative beta cell area was 1.48 %, 1.07 %, 0.92 % (vs NGT,  $P = 0.003$ ) and 0.52 % (vs NGT,  $P < 0.001$ ) in NGT, IGT, NDM and LDM, respectively. The numbers of single beta cells and clusters, defined as single insulin-positive cell and a unit comprising three cells or fewer insulin-positive cells, both of which did not form part of larger islet structures, were 2.0, 3.9 (vs NGT,  $P = 0.031$ ), 4.0 (vs NGT,  $P = 0.016$ ), and 1.5 (/5 mm<sup>2</sup>), respectively. On the other hand, the mean areas of one islet excluding single beta cells and clusters were 11756  $\mu\text{m}^2$ , 12847  $\mu\text{m}^2$ , 11402  $\mu\text{m}^2$ , 11374  $\mu\text{m}^2$ , respectively, with no significant difference among the groups, indicating that the hypertrophy

of islet did not occur in each group. The percentage of the duct cells positive for insulin, indicating beta cell neogenesis, was 0.59 %, 0.96 % (vs NGT,  $P = 0.018$ ), 0.72 % and 0.26 %, respectively. The percentage of beta cells positive for somatostatin, indicating immature beta cells during neogenesis, was 0.58 %, 1.14 % (vs NGT,  $P < 0.001$ ), 1.24 % (vs NGT,  $P = 0.014$ ) and 0.36 %, respectively. The percentage of beta cells positive for glucagon, also indicating immature beta cells during neogenesis, was 0.48 %, 0.73 % (vs NGT,  $P = 0.030$ ), 0.86 % (vs NGT,  $P < 0.001$ ), and 0.34 %, respectively. The percentage of beta cells positive for Ki67, indicating beta-cell replication, was 0.07 %, 0.10 %, 0.12 % and 0.04 %, respectively, with no significant difference among the groups. The percentage of beta cells positive for TUNEL, indicating beta-cell apoptosis, was 0.02 %, 0.03 %, 0.06 % and 0.12 % (vs NGT,  $P = 0.011$ ), respectively.

**Conclusion:** The relative beta cell areas decreased and beta cell apoptosis increased in the development from NGT to IGT, NDM, LDM. Beta cell replication did not increase, but beta cell neogenesis increased in IGT and NDM compared with NGT, indicating that beta-cell neogenesis is more important than replication as mechanism of compensation for decrease of beta cell mass. LDM had lower incidence of beta cell neogenesis, suggesting that this mechanism does not work after the long duration of type 2 diabetes.

*Supported by: JSPS, MHLW*

## PS 019 Islet cell development and generation II

424

### Is the side population of the human pancreas a source for adult stem cells?

P. Augstein<sup>1,2</sup>, T. Loudovaris<sup>3</sup>, E.M. Bandala Sanchez<sup>2</sup>, G. Naselli<sup>2</sup>, L. Lee<sup>2</sup>, K. Rogers<sup>2</sup>, W.J. Hawthorne<sup>4</sup>, F. Vaillant<sup>2</sup>, P. Heinke<sup>1</sup>, L.C. Harrison<sup>2</sup>;

<sup>1</sup>Institute of Diabetes, Karlsburg, Germany, <sup>2</sup>The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, <sup>3</sup>The St. Vincent's Institute of Medical Research, Melbourne, Australia, <sup>4</sup>Westmead Hospital, University of Sydney, Australia.

**Background and aims:** The 'side population' (SP) is characterized by the ability to efflux the fluorescent dye Hoechst 33342 and is visualized as a tail from the main population (non-SP) by UV excitation. In rodent models, the SP of several tissues including bone marrow and liver is enriched for adult stem cells. Our aim was to identify the SP and characterize its phenotype and colony-forming potential (CFP) in the adult human pancreas.

**Materials and methods:** Pancreata were obtained, with informed consent from next-of-kin, from heart beating, brain dead donors and processed by a modified Ricordi method of intraductal infusion and digestion with collagenase, followed by fractionation on a continuous Ficoll density gradient and a COBE cell processor. Non-islet pancreatic digest from 30 donors was cultured short-term and stained with Hoechst 33342 to identify the SP. For phenotyping, Hoechst-labeled cells were stained with antibodies to haematopoietic, mesenchymal and epithelial stem cell markers. Antibodies to islet (Hp1), acinar (Hpx2), large ductal (Hpd1) and all ductal (CA19-9) cells were used to identify the pancreatic cell type. Cells were analysed with a BD LSR II-W flow cytometer. Wilcoxon signed-rank test was used to test the level of statistical significance. CFP was evaluated in a 2D-assay with irradiated fibroblasts as feeder cells after sorting SP and non-SP cells in a BD FACSAria C.

**Results:** SP cells were detected in the non-endocrine digest of all donor pancreata and their proportion was independent of donor sex, age and BMI. A low proportion of SP and non-SP cells expressed the haematopoietic markers CD117 and CD45. The mesenchymal markers CD44 and CD29 were highly expressed on both SP and non-SP cells. Expression of CD166, CD90 and CD31 was similar on SP and non-SP cells. The proportion of non-SP cells expressing CD34 was higher than SP cells ( $p=0.034$ ). The epithelial marker EpCam was detected on nearly all SP and non-SP cells. Similarly, expression of CD24, CD49f and Trop-2 was similar on SP and non-SP cells. However, the proportion of cells expressing CD133 ( $p=0.001$ ) and CD26 ( $p=0.0001$ ) was increased in the SP. The islet cell surface antigen Hpi1 was detected at low frequency ( $p=0.281$ ) on SP and non-SP cells. Cells expressing the exocrine surface antigen Hpx2 were enriched in the non-SP ( $p=0.001$ ). However, the proportion of cells expressing the ductal markers Hpd1 ( $p=0.001$ ) and CA19-9 ( $p=0.008$ ) was increased in the SP. To evaluate CFP, SP and non-SP cells were sorted after exclusion of islet (HPi1-) and haematopoietic (CD45-, glycophorin-, CD31-) cells. After 2 weeks in culture, CFP in SP cells (0.29/100 cells) was greater than in non-SP cells (0.05/100 cells).

**Conclusion:** We confirm the presence of SP cells in the adult human pancreas and demonstrate for the first time that they are enriched for cells expressing the ductal marker CD133 and for CFP. These findings are consistent with the notion that SP cells are a reservoir of stem cells in the adult pancreas.

*Supported by: National Health and Medical Research Foundation of Australia; JDRF; EFSO*

425

### Pancreatic duct glands - a stem cell niche in the pancreas?

B. Gier<sup>1</sup>, A.E. Butler<sup>1</sup>, A.V. Matveyenko<sup>1</sup>, D. Kirakossian<sup>1</sup>, S.M. Dry<sup>1</sup>, M.A. Atkinson<sup>2</sup>, P.C. Butler<sup>1</sup>;

<sup>1</sup>Medicine/ Endocrinology, David Geffen School of Medicine, Los Angeles,

<sup>2</sup>Pathology, University of Florida, Gainesville, USA.

**Background and aims:** It is unknown if there is a pancreatic tissue stem cell niche. The pancreatic duct gland (PDG) compartment has been proposed to have this role harbouring both exocrine duct like cells and a minority of endocrine cells. Tissue specific stem cells are typically anatomically crypt like, harbour cells expressing early developmental markers relevant to the organ of interest and have transit amplifying zones of cells with a capacity for in-



creased replication that is amplified under conditions of inflammation in the client organ. Acute tissue damage thus prompts tissue repair while chronic organ damage may induce dysplasia. We sought to further examine PDGs in humans and relevant rodent models to determine if these properties of pancreatic stem cell niche are met, and if they are induced to proliferation by beta cell apoptosis in type 2 diabetes (T2DM).

**Materials and methods:** PDGs were isolated by laser microdissection of frozen pancreatic sections from humans with T2DM, non diabetic controls and in rats. RNA was isolated from captured cells, and after RNA denaturation and reverse transcription, real-time quantitative PCR was performed to identify expression of genes of interest. Immunohistochemistry of fixed tissue was used to complement RNA expression studies. The frequency of PDG replication was assessed by Ki67 immunostaining.

**Results:** Expression markers and transit amplifying zone of a stem cell niche? PDGs in humans and rats express the developmental genes *Nestin* and *Hes-1* as well as early pancreatic progenitor markers such as *Sox-9* and *Pdx-1*. PDGs also express *Ngn3*, the transcription factor required to direct progenitor cells towards an endocrine lineage. Moreover, a subset of PDG cells express *Lgr5* and *Aldh*, both specific stem cell markers in stem cell crypts of ileum and colon. Also, consistent with a stem cell transit amplifying zone, PDGs (humans and rats) have zones of increased replication (Ki67) compared to the rest of the pancreas. *Activation by inflammation in client tissue compartment?* PDG replication is increased in humans with T2DM compared to non diabetic controls (2.1% vs 0.8%). To establish if this is prompted by beta cell apoptosis or the metabolic sequelae of beta cell failure we quantified PDG replication in the human IAPP transgenic rat model of type 2 diabetes (HIP rat) compared to wild type (WT) rats. PDG replication was increased (7.9% vs 4.3%) in HIP rats before the onset of hyperglycemia but coincident with increased beta cell apoptosis. Moreover, chronic glucose infusion induced hyperglycemia in WT rats did not increase PDG replication. *Cell types of client organ?* The majority of cells in PDGs (humans and rats) have an exocrine duct cell phenotype but a minority (~1%) are endocrine cells, expressing insulin and/or glucagon.

**Conclusion:** The PDG compartment of the pancreas bears the anatomic and cellular molecular expression profile of a tissue stem cell compartment. Moreover PDGs undergo proliferation in response to beta cell apoptosis, consistent with attempted regeneration. Since PDGs appear to provide new cells in proportion to the epithelia cell types in pancreas (~1% endocrine, ~99% exocrine), chronic stimulation of this cell stem cell niche by beta cell apoptosis may induce exocrine dysplasia rather than effective beta cell regeneration. This may provide an explanation for increased pancreatic dysplasia (chronic pancreatitis and pancreatic cancer) in T2DM.

Supported by: DFG, NIH, Kompaniez Foundation, Hillblom Foundation

## 426

### Involvement of heparan sulfate 6-O-sulfotransferase isoform-1 in the mouse beta cell proliferation during pregnancy

I. Takahashi, T. Sato, F. Sato, K. Ohashi, K. Nata;

Dept of Medical Biochemistry, Iwate Medical University, Yahaba, Japan.

**Background and aims:** Maternal pancreatic islet proliferation in pregnancy is one of the interesting phenomena for  $\beta$ -cell expansion in physiological conditions. However, the molecular mechanisms underlying these events are only partially understood. Heparan sulfate (HS) is linear polysaccharides consisting of repeating disaccharide unit backbone onto which is superimposed a complex pattern of modifications, most notably addition of sulfate groups. The produced polymorphic sulfated sequence motifs are responsible for binding to a variety of signaling molecules and modulating their biological functions. Recently, we found that HS is localized exclusively around  $\beta$ -cells in islets of adult mice and required for islet morphogenesis,  $\beta$ -cell proliferation and insulin secretion. Furthermore, we found that the sulfate groups of HS, in particular the 3-O-sulfate modification generated by HS 3-O-sulfotransferase isoform-1, are necessary for maintaining normal glucose-induced insulin secretion (GIIS). The aim of this study is to clarify the effect of the modification in HS including sulfation for  $\beta$ -cell function and proliferation during pregnancy.

**Materials and methods:** Islets of Langerhans from C57BL/6J female mice were isolated from non-pregnant and pregnant (gestational days 13.5 [G13.5]) mice. Quantitative RT-PCR was used for analyzing mRNA expression of HS synthesis and modification enzymes and components of several signaling pathways. Expression of HS 6-O-sulfotransferase isoform-1 (Hs6st1) was silenced and GIIS and cell proliferation were examined in MIN6, insulin-secreting mouse insulinoma cell line.

**Results:** The HS synthesis and modification enzymes, including Ext/Extl gene family, N-deacetylase/N-sulfotransferases, Glucuronyl C5-epimerase (Glce), Hs6st1 and HS 3-O-sulfotransferases were detected in islets and MIN6 cells. Among these genes, increase of expression of Glce, Hs6st1 and HS 3-O-sulfotransferase isoform-3B1 during pregnancy was detected (fold change 3- to 4-fold) by real-time PCR analysis of islets mRNA isolated from non-pregnant and pregnant mice at G13.5. Silencing of a Hs6st1, one of these upregulated enzymes, by RNA interference reduced MIN6 cell proliferation to 77% of control treatment ( $p < 0.001$ ). On the other hand, GIIS was unaffected in MIN6 with the RNA interference on Hs6st1. We determined by RT-PCR the expression pattern of the components of signaling pathways downstream of HS, especially these pathways affected by 6-O-sulfate modification: signaling pathway of fibroblast growth factor (Fgf), Notch, Hedgehog, Wnt and Transforming growth factor  $\beta$ . The mRNA levels of Fgf receptor (Fgfr1 and Fgfr2, the Fgf signaling components), were increased in Hs6st1 silenced MIN6 when compared to the control (1.7- and 1.3-fold, respectively).

**Conclusion:** Our data suggest that 6-O-sulfate groups modified by Hs6st1 play important role(s) in the adaptive  $\beta$ -cell proliferation during pregnancy. Our result also suggests Fgf signaling components including Fgfr1 and 2 may be involved in the signaling pathway in  $\beta$ -cell proliferation downstream of HS.

## 427

### Increased beta cell replication but impaired beta cell neogenesis and deficient beta cell mass expansion in response to 90% pancreatectomy and to gastrin treatment in aged rats

N. Tellez<sup>1,2</sup>, M. Vilaseca<sup>1,2</sup>, E. Montanya<sup>1,2</sup>;

<sup>1</sup>Lab. Diabetes and Experimental Endocrinology, IDIBELL-University of Barcelona, L'Hospitalet de Llobregat, <sup>2</sup>CIBERDEM, Barcelona, Spain.

**Background and aims:**  $\beta$ -cell replication reduction with age may preclude  $\beta$ -cell mass expansion in response to the increased metabolic demand. However, the adaptive stimulation of  $\beta$ -cell neogenesis in aged pancreas has not been explored. In a recent study we found that gastrin treatment stimulated  $\beta$ -cell neogenesis, increased  $\beta$ -cell mass and improved the metabolic outcome after subtotal pancreatectomy (Px) in young rats. The aim of our study was to investigate the  $\beta$ -cell regeneration potential of aged rats exposed to Px and gastrin. **Materials and methods:** 1 and 12 months old Wistar rats underwent 90%-Px and were treated from the day of surgery with [15Leu] gastrin-17 (150  $\mu$ g/kg  $\cdot$  12h, Px+G, n=13) or with vehicle (Px+V; n=13). A group of sham-operated rats treated with vehicle was included for each age (S+V; n=10). Pancreatic remnants were harvested on day 14 upon Px and  $\beta$  (insulin), acinar (amylase) cell mass (morphometry) and replication (BrdU) were determined. Beta cell size, beta cell apoptosis (TUNEL) and indirect signs of  $\beta$ -cell neogenesis (scattered extra-islet beta cells) were additionally evaluated.

**Results:** Ninety percent Px induced pancreas regeneration in both young and aged rats shown by the increased pancreas weight 14 days after Px (Young rats, S+V: 83 $\pm$ 4.9mg; Px+V: 119 $\pm$ 7.3mg;  $p < 0.05$ ; aged rats, S+V: 152 $\pm$ 9.35mg; Px+V: 212 $\pm$ 15.4mg;  $p < 0.05$ ). Consistent with previous results, Px young rats showed increased  $\beta$ -cell mass (S+V: 0.37 $\pm$ 0.04mg; Px+V: 0.79 $\pm$ 0.12mg;  $p < 0.05$ ), and neogenesis (S+V: 0.28 $\pm$ 0.02%; Px+V: 0.37 $\pm$ 0.04%;  $p < 0.05$ ) that were further enhanced by gastrin treatment ( $\beta$ -cell mass: 1.40 $\pm$ 0.18mg;  $p < 0.05$  vs Px+V;  $\beta$ -cell neogenesis: 0.54 $\pm$ 0.07%;  $p < 0.05$  vs Px+V). Moreover, Px+G rats showed improved metabolic evolution compared to Px+V (day 14: 109 $\pm$ 3.84 mg/dl vs 124 $\pm$ 5.76 mg/dl;  $p < 0.05$ ).  $\beta$ -cell replication and apoptosis were similar among groups and individual beta cell size was similarly increased in both Px groups. In aged rats, both Px groups developed hyperglycemia (day 14; Px+V: 275 $\pm$ 87.8 mg/dl, Px+G: 282 $\pm$ 82.9 mg/dl). Acinar and  $\beta$ -cell replication were stimulated upon Px (acinar cell replication: S+V: 0.10 $\pm$ 0.02%; Px+V: 1.12 $\pm$ 0.14%; Px+G: 0.9 $\pm$ 0.14%;  $p < 0.05$  S+V vs Px groups;  $\beta$ -cell replication: S+V: 0.03 $\pm$ 0.03%; Px+V: 0.53 $\pm$ 0.11%, Px+G: 0.78 $\pm$ 0.19%;  $p < 0.05$  S+V vs Px groups). Accordingly, acinar cell mass was increased in Px rats (S+V: 121 $\pm$ 8.8mg; Px+V: 175 $\pm$ 16.5mg; Px+G: 179 $\pm$ 9.6mg;  $p < 0.05$  S+V vs Px groups). However,  $\beta$ -cell mass was similar among groups (S+V: 1.8 $\pm$ 0.35mg, Px+V: 1.46 $\pm$ 0.36mg, Px+G: 1.45 $\pm$ 0.20mg), and  $\beta$ -cell neogenesis was not increased in Px aged rats (S+V: 0.27 $\pm$ 0.04%; Px+V: 0.27 $\pm$ 0.03%; Px+G: 0.23 $\pm$ 0.02%).

**Conclusion:** In aged rats, acinar cell regeneration was increased upon Px leading to acinar cell mass expansion. In contrast,  $\beta$ -cell mass expansion was impaired despite the increased  $\beta$ -cell replication.  $\beta$ -cell neogenesis, which was significantly stimulated in young rats, was impaired in response to Px and gastrin treatment in aged rats, suggesting that  $\beta$ -cell neogenesis plays a role on adult  $\beta$ -cell mass expansion.

Supported by: ACD, SED, CIBERDEM from ISCIII

## 428

**Neurotransmitters of the parasympathetic system regulate beta cell regeneration in 90% pancreatectomised rat**

A. Ilias, M. Alawieh, M. Ah Kioon, B. Portha, J. Movassat;  
University Paris Diderot/ CNRS, Paris, France.

**Background and aims:** Pancreatic beta cells retain the capacity to regenerate in the context of cell deficiency. To date, the regulation of beta cell regeneration by the neurotransmitters of the autonomous nervous system is poorly documented. Our aim was to decipher the individual role of the parasympathetic neurotransmitters: Acetylcholine and Gastrin Releasing Peptide (GRP) in the regulation of pancreatic beta cell regeneration following 90% pancreatectomy (Px) in adult rat.

**Materials and methods:** We developed an approach based on the specific inactivation of receptors of these neurotransmitters (Muscarinic M3 receptor for acetylcholine, GRP receptor) by the means of antisense morpholino oligonucleotides (As Mo). Immediately after 90% Px, a specific As Mo against each of the above receptors were injected within the remnant pancreas of a group of rats. Another group of rats was treated with non specific standard morpholino (Std) and served as control. 8h, 48h or 7days after surgery, animals were sacrificed and remnant pancreases were removed and processed for immunohistochemistry. Beta cell proliferation was assessed by BrdU incorporation method and the beta cell mass was evaluated by morphometry.

**Results:** Our data show that downregulation of M3 and GRP receptors dramatically reduced beta cell proliferation 48h after surgery. The beta cell mass measured at 48h and 7 days post-surgery was also significantly reduced at least partly due to the decreased beta cell proliferation.

**Conclusion:** In conclusion we reveal that among neurotransmitters of the parasympathetic nervous system, acetylcholine and GRP play a significant role in the regulation of the beta cell mass regeneration after 90% pancreatectomy.

*Supported by: EFSD/Amylin grant, SFD*

## 429

**Rho-kinase and TGF- $\beta$ 1 inhibition delay dedifferentiation of pancreatic exocrine cells in culture**

K.R. Muir, M.J. Lima, K. Docherty;  
University of Aberdeen, UK.

**Background and aims:** A cell based therapy is seen by many as the best hope of a cure for type 1 diabetes mellitus. When placed in standard culture conditions pancreatic islets rapidly dedifferentiate to take on a mesenchymal stem cell phenotype losing the ability to produce insulin and other endocrine hormones. A similar process takes place with exocrine pancreatic cells losing amylase production after dedifferentiation. The exocrine fraction of the pancreas holds great potential in reprogramming towards beta cells due to plentiful supply and similarities in embryological development. Strategies for prevention of this dedifferentiation process may involve the targeting of selected cell signalling pathways. We propose that the inhibition of Rho kinase in combination with TGF $\beta$ 1 inhibition can slow down this process of epithelial to mesenchymal transition and lead to cells retaining epithelial morphology and function for longer in culture.

**Materials and methods:** An exocrine enriched pancreatic cell population left over from islet transplantation was used in the experiments. Immediately after arrival the cells were plated on 6 well plates at 175 000 clusters per well with RPMI medium + 10% fetal bovine serum and left for 48 hours to attach. At this point they were incubated with either a Rho Kinase inhibitor (Y27632), a TGF $\beta$ 1 inhibitor (SB431542), or a combination of both. The cells were harvested after 5 days and gene expression of endocrine, exocrine, epithelial and mesenchymal markers quantified by RTqPCR. These results were also compared to samples harvested at 48hours prior to treatment.

**Results:** Following treatment with both Y27632 and SB431542 expression of amylase showed a 334% increase compared to untreated control and 168% increase compared to baseline at 48 hours in culture. Insulin showed a 571% increase compared to control but a decrease of 69% compared to baseline. Glucagon expression was 535% higher than control and 4% lower than baseline. Epithelial marker E-cadherin showed a 29% increase compared to control and 12% increase compared to baseline. All these markers were elevated at a statistically significant level compared with untreated controls. The mesenchymal marker vimentin remained statistically unchanged throughout. A greater effect was seen when Y27632 and SB431542 were used in combination rather than individually.

**Conclusion:** The results suggest that the addition of Y27632 and SB431542 to exocrine enriched pancreatic cells in the initial stages of culture may slow down the epithelial to mesenchymal transition that rapidly ensues. This holds promise as maintaining an epithelial morphology may result in cells that are more amenable to reprogramming towards an endocrine lineage. Further work is required to determine whether these inhibitors have the potential to reverse fully dedifferentiated cells. If this is possible dedifferentiated cells could be expanded and later redifferentiated providing a near unlimited supply of exocrine material for reprogramming.

*Supported by: STMTI*

## 430

**Expression of IBCAP (intestine-derived beta cell augmenting promoter), an intestine specific secretory factor, induces insulin positive cells in the liver**

T. Yokoo<sup>1</sup>, K. Watanabe<sup>2</sup>, K. Tada-Iida<sup>2</sup>, H. Suzuki<sup>2</sup>, H. Shimano<sup>2</sup>, M. Kawakami<sup>3</sup>, N. Yamada<sup>2</sup>, Y. Okazaki<sup>1</sup>, H. Toyoshima<sup>1,3</sup>;

<sup>1</sup>Division of Functional Genomics & Systems Medicine, Research Center for Genomic Medicine, Saitama Medical University, Hidaka, <sup>2</sup>Department of Internal Medicine, Metabolism and Endocrinology, Faculty of Medicine, University of Tsukuba, <sup>3</sup>1st Department of Comprehensive Medicine, Saitama Medical Center, Jichi Medical University, Saitama, Japan.

**Background and aims:** IBCAP is an intestine-specific secretory protein with incretin-like activity, discovered in our laboratory. Adenoviral expression of IBCAP was shown to increase the amount of pancreatic  $\beta$ -cells, and improves the glycemic control of STZ-treated diabetic mice. Also, transgenic mice over-expressing IBCAP was demonstrated to have increased amount of pancreatic  $\beta$ -cells, whereas gene-knockouts of the factor resulted in a marked decrease of pancreatic islets. Our findings strongly suggested IBCAP as a major player in the control of the amount of pancreatic islets and  $\beta$ -cells. In the current study, we investigate the  $\beta$ -cell transdifferentiation function of IBCAP in the liver.

**Materials and methods:** STZ treated diabetic mice were randomized based on body weight, glucose, insulin and HbA1c. Liver samples and paraffin-embedded liver sections of STZ-treated diabetic mice infected with recombinant adenovirus intravenously over-expressing either IBCAP or a control protein GFP were analyzed. The microscopic examination of insulin expression in the liver was analyzed by immunostaining.

**Results:** 7 days after injection of IBCAP-expressing adenovirus into the tail vein of STZ-diabetic mice diabetic condition was improved and insulin expression in the liver was detected by immunostaining. Insulin positive cells were demonstrated in the liver of STZ treated type 1 diabetes model mice infected with IBCAP expressing adenovirus, but not in the mice infected with a control.

**Conclusion:** Our results demonstrated that expression of IBCAP can induce insulin-positive cells in the liver. We assume that IBCAP have an activity to induce the differentiation of the progenitor cells to insulin producing  $\beta$ -cells. We are currently working on this hypothesis.

*Supported by: The Ministry of Education, Culture, Sports, Science and Technology of Japan*

## 431

**Defective proliferative and differentiation properties of human pancreatic islet derived mesenchymal cells from type 2 diabetic donors**

F.A. Grieco<sup>1,2</sup>, I. Spagnuolo<sup>1,2</sup>, A. Patti<sup>1,2</sup>, G. Sebastiani<sup>1,2</sup>, M. Bugliani<sup>3</sup>, L. Marselli<sup>3</sup>, P. Marchetti<sup>3</sup>, F. Dotta<sup>1,2</sup>;

<sup>1</sup>University of Siena, <sup>2</sup>Fondazione Umberto Di Mario ONLUS-Toscana Life Science, Siena, <sup>3</sup>University of Pisa, Italy.

**Background and aims:** It has been established that both in Type 1 and Type 2 diabetes (T2D) there is a significant loss of insulin-producing beta cells and consequently decreased functional beta cell mass. *In vitro* generation of insulin-producing cells from stem/progenitor cells is crucial to develop a proper cell-therapy for diabetes. Previously, we have obtained *in vitro* human pancreatic islet derived mesenchymal (hPIDM) cells able to de-differentiate from native islets, to proliferate and to re-acquire a pancreatic endocrine phenotype. Consequently, we aimed at investigating whether islets derived from T2D patients retain above mentioned properties observed in non diabetic donors.

**Materials and methods:** Human Pancreatic Islet-Derived Mesenchymal (hPIDM) cells were obtained by plating human pancreatic islets from T2D

donors in RPMI 1640 medium containing 10% FBS. For differentiation into a pancreatic endocrine phenotype, cells were cultured for 21 days in serum-free RPMI 1640 medium containing BSA, insulin, transferrin and sodium selenite (SFM). Proliferating and differentiated hPIDM cells were characterized by Real-Time-PCR and immunofluorescence analysis for expression of endocrine and mesenchymal markers. Glucose-induced C-peptide secretion was evaluated by RIA test.

**Results:** During de-differentiation, Real-Time-PCR after 15 days and after 6 passages of culture revealed a significant loss ( $p < 0.05$ ) of pancreatic endocrine genes (i.e. insulin, glucagon, somatostatin, Pdx-1) and an up regulation of mesenchymal genes (vimentin, nestin), compared to native islets. Double immunofluorescence analysis for insulin, nestin, vimentin, smooth muscle  $\alpha$ -actin and Ki-67, after 3, 6, 11 and 15 days of culture, showed disappearance of insulin positive cells at a faster rate in T2D patients vs control donors (3 days vs 10 days). Moreover, proliferative marker Ki67 appeared later in hPIDM cells obtained from T2D patients, and the growth curve showed reduced proliferative properties in such cells vs hPIDM cells from non-diabetic donors. After culture in SFM medium, cells re-acquired a pancreatic endocrine phenotype at gene expression level by real Time PCR; however, differently from what observed in control donors, cells obtained from T2D patients resulted negative for insulin, glucagon and somatostatin by immunofluorescence, nor released C-peptide after glucose stimulation.

**Conclusions:** Taken together these data show that islet cells from T2D patients are able to de-differentiate into hPIDM cells, which, however, show impaired proliferation and defective properties in terms of re-differentiation into a pancreatic endocrine phenotype, thus uncovering a “regenerative” defect in T2D. Such cells from may represent an *in vitro* model to test the capacity of new anti-diabetic drugs to induce/modulate beta cell regeneration. *Supported by: Tuscany Region*

## 432

### Co-culture of islets with mesenchymal stem cells in direct contact configurations improves islet function *in vitro*

P.K. Dhadda, C.L. Rackham, S.J.S. Simpson, P.M. Jones;  
Diabetes and Nutritional Sciences, School of Medicine, King's College London, UK.

**Background and aims:** Co-transplantation of islets with mesenchymal stem cells (MSCs) has been shown to enhance the outcome of islet transplantations *in vivo*, which may be a result of improved islet function. This study aimed to determine whether MSCs improve islet function when co-cultured with islets in different direct contact configurations *in vitro*.

**Materials and methods:** Kidney MSCs and pancreatic islets were isolated from C57BL/6 mice. Real time imaging sequences of fluorescently labelled MSCs or non-labelled MSCs, and non-labelled islets in suspension co-culture were captured to assess the interactions between MSCs and islets over a 72 hour time period. Islets were also cultured alone (IA), in suspension co-culture (SC) or cultured directly upon a monolayer of MSCs (MC) for 48 hours, and subsequently analysed for insulin content and glucose-stimulated insulin secretion by radioimmunoassay.

**Results:** *Real time imaging:* Real time imaging revealed that MSCs migrated rapidly towards and adhered to the surface of islets in a largely random manner, forming a mantle to increase islet size. Formation of the MSC-islet composite structures took place in a time dependent manner, with a maximal increase in islet diameter being measured after 36 hours ( $22 \pm 3\%$ ,  $n=3$ ,  $p < 0.05$  versus control). No further significant increase in islet diameter was seen after this time point. Real time fluorescence microscopy of islets co-cultured with fluorescent Qtracker-labelled MSCs revealed that MSCs formed an intact mantle around the perimeter of the islets and also penetrated and migrated into the islet structure. *Co-culture:* Islets cultured alone for 48 hours were able to secrete insulin in response to both intermediate (10mmol/l) and high (20mmol/l) concentrations of glucose (2mmol/l glucose:  $0.04 \pm 0.02$  ng/islet/h; 10mmol/l:  $0.08 \pm 0.03$ ; 20mmol/l:  $0.13 \pm 0.03$ ,  $n=9$ ,  $P < 0.05$ ). Glucose stimulated insulin secretion was enhanced when islets were co-cultured either as SC (2mmol/l:  $0.04 \pm 0.01$  ng/islet/h; 10mmol/l:  $0.15 \pm 0.08$ ; 20mmol/l:  $0.42 \pm 0.12$ ,  $n=9$ ) or as MC (2mmol/l:  $0.14 \pm 0.05$  ng/islet/h; 10mmol/l:  $0.89 \pm 0.22$ ; 20mmol/l:  $2.33 \pm 0.5$ ,  $p < 0.05$ ,  $n=9$ ) compared to islets alone. Both co-culture configurations resulted in elevated islet insulin content when compared to islets cultured alone (IA:  $9.7 \pm 3.0$  ng/islet; SC:  $13.9 \pm 0.7$ ; MC:  $23.8 \pm 7.5$ ,  $n=3$ ).

**Conclusion:** Both *in vitro* co-culture configurations were able to enhance islet function, which may have important implications for clinical islet transplantation practices. Pre-culturing islets on a monolayer of MSCs immediate-

ly after isolation, but prior to clinical transplantation, may serve as a superior option to culturing islets alone, while the formation of islet-MSC composites may provide an attractive option for the co-localised delivery of both cell types for intraportal delivery.

*Supported by: MRC, DUK*

## 433

### Beta cell adaptation is topologically heterogeneous throughout the pancreas

J.H. Ellenbroek<sup>1</sup>, H.A. Töns<sup>1</sup>, N. de Graaf<sup>1</sup>, C.J.M. Loomans<sup>2</sup>, M.A. Engelse<sup>1</sup>, H. Vrolijk<sup>3</sup>, T.J. Rabelink<sup>1</sup>, F. Carlotti<sup>1</sup>, E.J.P. de Koning<sup>1,2</sup>;  
<sup>1</sup>Department of Nephrology, Leiden University Medical Center, <sup>2</sup>Hubrecht Institute, Utrecht, Netherlands, <sup>3</sup>Department of Molecular Cell Biology, Leiden University Medical Center, Netherlands.

**Background and aims:** Beta cells adapt to an increased insulin demand by enhancing insulin secretion via increased beta cell function and/or increased beta cell number. Insight into the mechanisms that control beta cell adaptation is important for developing therapies that can preserve or enhance beta cell mass. It is unknown whether there are regional differences in beta cell adaptation throughout the pancreas. We investigated beta cell adaptation in a model of high-fat diet (HFD)-induced insulin resistance in mice.

**Materials and methods:** C57BL/6 mice were fed a HFD or control diet for 6 weeks. Glucose and insulin tolerance tests were performed at the end of the study. BrdU was injected twice daily to label proliferating cells one week before sacrifice. The pancreas was divided in a duodenal (DR), gastric (GR) and splenic (SR) region and taken for either histology or islet isolation followed by a glucose-induced insulin secretion test. The capacity of islets from the three regions to adapt in an extrapancreatic location was assessed by transplantation of the untreated isolated islets under the kidney capsule of diabetic mice. BrdU was used to label proliferating cells (starting from day 3 post transplantation) and blood glucose levels were monitored for 10 days, after which the islet graft was taken for histology.

**Results:** As expected, HFD mice showed decreased insulin sensitivity compared to control mice. However, only SR islets showed increased  $\beta$ -cell proliferation (BrdU+/Ins+ cells  $5.8 \pm 1.2$  (HFD) vs.  $3.3 \pm 0.4\%$  (control),  $p < 0.05$ ). Furthermore, glucose-induced insulin secretion was increased twofold from isolated SR islets after HFD compared to DR and GR islets (insulin/DNA  $0.39 \pm 0.02$  (SR),  $0.23 \pm 0.03$  (DR),  $0.25 \pm 0.03$  (GR),  $p < 0.05$ ). Transplantation of islets from the SR, GR and DR in syngeneic diabetic mice led to a similar decrease in hyperglycaemia and there was no difference in beta cell proliferation (BrdU+/Ins+ cells  $24.5 \pm 2.2$  (DR),  $21.5 \pm 3.1$  (GR),  $25.0 \pm 1.7\%$  (SR),  $p = 0.53$ ) indicating that the regional peri-islet microenvironment of the pancreas is important for differential adaptive responses.

**Conclusion:** HFD-induced insulin resistance leads to topologically heterogeneous beta cell adaptation and is most prominent in the splenic region of the pancreas. This topological heterogeneity in beta cell adaptation is the result of extrinsic factors present in the islet microenvironment. Investigation of these regional differences may lead to the identification of novel factors involved in beta cell regeneration.

*Supported by: The DCTI consortium including the Dutch Diabetes Research Foundation*



## PS 020 Islet transplantation

### 434

#### Evolution of diabetes complications 5 years after islet transplantation (IT) with the Edmonton immunosuppressive regimen

**D. Quintin<sup>1</sup>**, J. Warin<sup>1</sup>, A.-S. Balavoine<sup>1</sup>, F. Defrance<sup>1</sup>, J. Kerr-Conte<sup>2</sup>, C. Noel<sup>3</sup>, F. Pattou<sup>4</sup>, M.-C. Vantyghem<sup>1</sup>;

<sup>1</sup>Endocrinology and Metabolism, Lille University Hospital, <sup>2</sup>Inserm U 859, Lille University Hospital, <sup>3</sup>Nephrology, Lille University Hospital, <sup>4</sup>Endocrine Surgery, Lille University Hospital, France.

**Background and aims:** Long-term benefit-risk ratio of IT remains poorly evaluated. The aim of this work was to determine evolution of diabetes complications 5 years after IT.

**Materials and methods:** 21/36 consecutive patients transplanted in a single center, had at least 5 years of follow-up. Their initial features were: duration of C-peptide negative diabetes: 28±9 years; age: 44±7 years; BMI: 23±2 kg/m<sup>2</sup>; 8/21 kidney-transplanted patients for 25±10 months. These 21 patients had systematic screening for macro and micro-angiopathy before IT, and yearly for 5 years after IT. Two “islet-alone (ITA)” and 2 “islet-after-kidney (IAK)” patients had lost their islet function at 5 years. Ten patients (48%) were insulin-independent (II), 8/10 with HbA1c ≤ 6.5%. Mean HbA1c level was 8.1±1.0% before vs. 7.0±1.0% 5 years after IT (p<0.01). Analyses were performed in intention-to-treat. There was no loss of follow-up.

**Results:** Mean level of cholesterol total (1.88±0.42 vs. 1.75±0.34 g/l), HDL (0.71±0.18 vs. 0.73±0.27 g/l), LDL (0.98±0.32 vs. 0.88±0.30 g/l), triglycerides (TG) (0.87±0.45 vs. 0.81±0.34 g/l) and mean BP (124±14/74±8 vs. 129±13/72±8 mmHg) did not differ before vs. after transplantation (patients treated with statins: 28% vs. 65% and with ≥1 antihypertensive drug: 38% vs. 75%). There were no clinical acute cardiovascular events during these 5 years. Before vs 5 years post IT, anomalies were detected on carotid (37% vs. 52%) and lower limb ultrasound (22 vs. 25% after exclusion of vascular calcifications), myocardial scintigraphy (19% vs. 24%). Four patients required coronary stenting. Retinopathy mildly worsened in 5/37 (14%) eyes in 21 patients (5 blind eyes). It remained stable or improved in 32/37 eyes (86%). Post-prandial glucose levels (p<0.001), mean glucose (MG) and standard deviation (SD) on CGMS (p<0.01) were higher in the worsened group compared to the stable one. The evolution of visual acuity did not vary significantly over time in the whole group, but differed between ITA and IAK (p<0.05), perhaps in relation to a higher frequency of cataract in IAK patients (p=0.056). Patients with cataract had a higher level of TG, BP (p<0.01) and cholesterol (p<0.05). Serum creatinine level, glomerular filtration rate assessed with MDRD and microalbuminuria did not vary significantly between 0 and 5 years. Creatinine and MDRD correlated negatively with GAD antibodies (respectively p<0.001 and 0.05), positively with tacrolimus (p<0.01) and triglyceride (p=0.01); microalbuminuria correlated with βscore (p<0.05), TG (p<0.01), and systolic BP (p<0.001). The means of motor and sensitive potential amplitudes (p<0.05) and sensitive nerve conduction (SNC)(p<0.01) improved between 0 and 5 years in the whole group, mainly in correlation with βscore for the sensitive parameters, and C-peptide, MG and TG for the amplitudes.

**Conclusion:** IT was not associated with acute clinical cardiovascular event in these 21 IT patients at the expense of systematic screening and treatment of silent coronaropathy. Kidney function and visual acuity remained stable. Sensitive nerve conduction improved, suggesting that despite immunosuppressive regimen, the Edmonton protocol is able to stabilize or even improve diabetes complications.

Clinical Trial Registration Number: NCT00446264 and NCT01123187

Supported by: PHRC, FEDER

### 435

#### Portal versus systemic venous drainage of pancreatic graft: the effect on glucose metabolism in pancreas and kidney transplant recipients

**T. Havrdova<sup>1</sup>**, F. Saudek<sup>1</sup>, P. Boucek<sup>1</sup>, T. Jedinakova<sup>1</sup>, K. Lipar<sup>2</sup>, M. Kocik<sup>2</sup>, J. Skibova<sup>3</sup>;

<sup>1</sup>Diabetes Center, Inst Clin Exp Medicine, Prague, <sup>2</sup>Transplant Center, Inst Clin Exp Medicine, Prague, <sup>3</sup>Statistical Department, Inst Clin Exp Medicine, Prague, Czech Republic.

**Background and aims:** Two different methods of graft venous drainage are used in pancreas transplantation: portal (PVD) and systemic (SVD). PVD

is considered as more physiologic due to similarity to venous outflow of the native pancreas. The aim of our study was to compare glucose metabolism in Type 1 diabetic recipients of kidney and pancreatic grafts with PVD versus SVD.

**Methods:** We examined 28 insulin-independent patients after simultaneous pancreas and kidney transplantation: 14 recipients with PVD of the pancreatic graft and 14 ones with SVD after a mean post transplant period of 1 year. All recipients had a stable good function of the kidney graft (mean serum creatinine level 103±19 [SD] μmol/l). Tacrolimus-based immunosuppression combined with mycophenolate mofetil or sirolimus was used in all patients. Fasting glycemia, insulin levels, HbA<sub>1c</sub>, a standard IVGTT with coefficient of glucose assimilation (K<sub>G</sub>) calculation and trough tacrolimus and sirolimus levels were assessed. Insulin sensitivity and production were evaluated using the homeostasis model assessment (HOMA-IR, HOMA-B). Total C-peptide and insulin secretions were calculated as areas under the curves from the serum levels during the IVGTT.

**Results:** PVD and SVD groups did not differ in age, BMI, duration of post transplant period, fasting C-peptide and insulin levels. We did not find any significant difference in response of IVGTT. In the PVD group 1 patient had an abnormal response to the glucose stimulus, 8 patients had an impaired glucose tolerance and 5 patients had a normal glucose tolerance. In the SVD group an abnormal response was present in none, the impaired glucose tolerance in 4 and the normal glucose tolerance in 10 recipients. Mean K<sub>G</sub> was 1.23±0.47 %/min. in the PVD group and 1.67±0.57 %/min. in the SVD group (p=0.12). The remaining results are shown in the following table. Trough levels of tacrolimus and sirolimus had no significant impact on any of the examined parameters.

**Conclusion:** Use of different types of venous drainage (of pancreatic graft) in Type 1 diabetic pancreas and kidney transplant recipients had no effect on glucose metabolism at one year post transplant.

	HbA1c DCCT (%)	Fasting glycemia (mmol/L)	HOMA-IR	HOMA-B	Total C-peptide secretion (pmol/ L/60min.)	Total insulin secretion (mIU/ L/60min.)
PVD group	5.41±0.32	4.89±0.55	1.17±0.82	19.0±16.9	93.3±31.0	1067±645
SVD group	5.69±0.27	4.7±0.72	2.64±2.26	51.6±46.4	96.3±31.0	1663±1136
Difference	p=0.09	p=0.57	p=0.1	p=0.07	p=0.86	p=0.22

Supported by: MZO 00023001

### 436

#### Is PTP1B involved in the protective effect of tungstate on transplanted islets grafts?

**H. Figueiredo<sup>1,2</sup>**, A. Garcia<sup>1,2</sup>, H.S.M. Farghaly<sup>3</sup>, R. Fernandez<sup>1,2</sup>, A. Novials<sup>1,2</sup>, R. Gomis<sup>1,2</sup>, R. Malpique<sup>1,2</sup>;

<sup>1</sup>Diabetes and Obesity, IDIBAPS-Hospital Clinic, Barcelona, Spain,

<sup>2</sup>CIBERDEM, Barcelona, Spain, <sup>3</sup>Faculty of Medicine, Assiut, Egypt.

**Background and aims:** Islet transplantation is considered a potentially curative treatment for type 1 diabetes, however its clinical application remains conditioned by post-transplantation challenges, such as inflammation and poor revascularization of the engrafted islets. Previous recent results by our group showed that after transplantation of pancreatic islets into the anterior chamber of the eye of diabetic-induced mice, treatment with tungstate (a phosphatase inhibitor) improved glycemic levels, increased islet graft revascularization and reduced cell death. Based on these observations, as well as the knowledge of the roles of the tyrosine phosphatase PTP1B in angiogenesis, apoptosis and insulin secretion, we hypothesize that the beneficial action of tungstate on the survival and function of engrafted islets may be mediated by PTP1B inhibition.

**Materials and methods:** Islets isolated from wild-type and PTP1B-knockout mice were transplanted into the anterior chamber of the eye of diabetic-induced mice (streptozotocin -treated 8 days before transplantation). The animals were divided into 6 groups: T+W - transplanted (T) with wild-type islets, treated with sodium tungstate (W; 0.5 mg/ml water); T - transplanted, non-treated with W; KO+W - transplanted with PTP1B-KO islets, W-treated; KO - transplanted with PTP1B-KO islets, non-treated with W; CTRL+W: non-transplanted, W-treated; CTRL - non-transplanted, non-treated with W. In vivo studies were performed during 25 days of treatment to evaluate glycemic levels, islet revascularization and cell viability. Post-mortem morphometric

analysis and functional studies were conducted on graft-containing eyes and pancreas. To complement in vivo findings, in vitro studies were performed by culturing isolated islets for 24 hours in medium enriched with tungstate or serum from diabetic animals treated with tungstate. Immunohistochemistry analyses were conducted on intact islets using endothelial and islet-cell markers as well as markers of cell proliferation and apoptosis.

**Results:** Our results showed, for T+W, KO+W and KO groups, decreased glycaemic levels 25 days after transplantation ( $279 \pm 48$ ,  $271 \pm 91$  and  $287 \pm 78$  mg/dl, respectively), when compared to the values obtained for the T, CTRL+W and CTRL groups ( $449 \pm 34$ ,  $478 \pm 162$  and  $548 \pm 52$  mg/dl, respectively). Furthermore, when islet vascular density was analysed after in vivo dextran injection, the T+W, KO+W and KO groups showed an increased value regarding the T group ( $0.028 \pm 0.002$ ,  $0.030 \pm 0.003$  and  $0.026 \pm 0.004$  versus  $0.016 \pm 0.003$  %). In accordance with these findings, morphometric analyses of the graft-containing eyes revealed an increased percentage of endothelial cells on islets from the T+W, KO+W and KO groups when compared with the T group. In parallel, a significant 6% increase ( $p < 0.01$ ) in the endothelium cells (CD31 staining) content was found for islets incubated in medium with serum from diabetic animals treated with tungstate as compared to islets incubated in medium with serum from tungstate non-treated diabetic mice.

**Conclusion:** Our results support the hypothesis that the protective action of tungstate on engrafted islets may be mediated by a mechanism dependent on PTP1B inhibition. Future work will focus on further unravelling of the molecular mechanism involved in these findings.

*Supported by: MICINN, Generalitat de Catalunya and CIBERDEM, Spain*

## 437

### Layer-by-layer nanoencapsulation of human islets

F. Syed, S. Krol, M. Bugliani, M. Masini, M. Suleiman, L. Marselli, F. Filipponi, U. Boggi, P. Masiello, P. Marchetti;  
Endocrinology and Metabolism, Metabolic Unit, University of Pisa, Italy.

**Background and aims:** Promising results are being reported in islet transplantation to cure subjects with type 1 diabetes. A major problem remains the need for chronic immunosuppression, that however could be potentially overcome if the islets are coated with biocompatible, semipermeable membranes to protect from immunological attacks. Whereas the use of macro- or microencapsulation techniques has given conflicting results, an attractive strategy involves the use of conformal islet nanocoating, with reduced volumes and the possibility of implantation into any suitable site. In this study we coated isolated human islets (HI) by a multilayer nanoencapsulation procedure and assessed several beta cell features.

**Materials and methods:** HI were isolated from non-diabetic cadaveric donors (age:  $63 \pm 8$  yrs, BMI:  $31.4 \pm 6.4$  kg/m<sup>2</sup>, mean blood glucose at ICU stay:  $141 \pm 50$  mg/dl) by enzymatic digestion and density gradient purification. Chitosan [(Poly(D-glucosamine) Deacetylated chitin)] and PSS [(Poly(styrenesulfonic acid sodium salt)] were dissolved (1 mg/ml) in M199 culture medium and kept at 37°C for 36 hours. HI were then incubated in each polymer for 7 min and nanoencapsulation was achieved by electrostatic binding of chitosan and PSS layer-by-layer. Morphology, morphometry, ultrastructure, viability and insulin secretion studies were then performed with non-encapsulated and nanoencapsulated islets cultured for 7 days.

**Results:** The layer-by-layer encapsulation procedure provided full coating of all the islets, as assessed by confocal and fluorescence microscopy examination after the use of chitosan labeled with FITC. Vital staining with propidium iodide showed  $\geq 90\%$  cell survival at all the experimental conditions; this was confirmed by electron microscopy, that also showed well maintained beta cell ultrastructure of the coated islet cells, with unchanged morphometry of intracellular organelles. Insulin secretion from coated islets was  $60 \pm 37$  uU/ml at 3.3 mmol/l glucose and increased to  $197 \pm 56$  uU/ml at 16.7 mmol/l glucose ( $p < 0.01$ ), with a stimulation index of  $5.0 \pm 3.7$  (all values not significantly different from those obtained with uncoated islets).

**Conclusion:** Therefore, isolated human islets were efficiently encapsulated by layer-by-layer nanocoating, with maintained morphological, survival and functional properties, providing a tool for experimental transplantation studies.

*Supported by: CariPisa Foundation*

## 438

### Free and encapsulated human pancreatic islets release insulin in response to hypotonicity in Ca<sup>2+</sup> independent manner

V. Štrbák<sup>1,2</sup>, Z. Bačovská<sup>1,2</sup>, G. Koláriková<sup>3</sup>, M. Orečná<sup>1</sup>, J. Oberholzer<sup>4</sup>, R. Hafko<sup>1</sup>, I. Lacík<sup>3</sup>;

<sup>1</sup>Inst. Exp. Endocrinology SAS, Bratislava, Slovakia, <sup>2</sup>Pathophysiology, Slovak Medical University, Bratislava, Slovakia, <sup>3</sup>Polymer Institute, SAS, Bratislava, Slovakia, <sup>4</sup>Department of Surgery, University of Illinois at Chicago, Chicago, USA.

**Background and aims:** We have previously shown that cell swelling induced by hypotonic medium reliably stimulates insulin secretion from rat pancreatic islets using unique signaling pathway including Ca<sup>2+</sup> independence and resistance to noradrenalin inhibition. The effect of cell swelling on insulin secretion from human islets has not been studied yet. This contribution shows this effect in post mortem isolated both free and encapsulated human pancreatic islets.

**Materials and methods:** Post mortem isolated islets were shipped from Chicago to Bratislava and encapsulated in sodium alginate-cellulose sulfate-poly(methyl-co-guanidine) microcapsules. Islets were incubated at basal and stimulated conditions either free or encapsulated within a semipermeable membrane, the latter as a part of our studies into immune protection of transplanted pancreatic islets by encapsulation. Free islets were also perfused with Ca<sup>2+</sup> containing or Ca<sup>2+</sup> depleted basal and stimulating media.

**Results:** Stimulation with 20 mM glucose or 30% hypotonicity induced insulin release from both free and encapsulated human islets (increase to 300 %,  $P < 0.001$  paired t-test between basal and stimulated group). Perfusion of free islets with Ca<sup>2+</sup> depleted medium revealed accentuated response to hypotonicity and depressed glucose-induced secretion.

**Conclusion:** Post mortem isolated human pancreatic islets, either free or encapsulated, release insulin in response to hypotonicity and glucose. Osmotically induced secretion is independent from extracellular Ca<sup>2+</sup>.

*Supported by: VEGA 2/0094/09, APVV 0235-06 and 0486-10, CENDO, Chicago Diabetes Project*

## 439

### Testing in vivo functionality of alginate-encapsulated pancreatic islets: advancing to the surgically-induced diabetic canine model

M. Ader<sup>1</sup>, R. Storrs<sup>2</sup>, J. Endres<sup>3</sup>, M. Lamb<sup>3</sup>, E.M. Paredes<sup>1</sup>, R.N. Bergman<sup>1</sup>, J.R.T. Lakey<sup>4</sup>;

<sup>1</sup>Cedars-Sinai Medical Center, Los Angeles, <sup>2</sup>Islet Sheet Medical LLC, San Francisco, <sup>3</sup>Dept. Surgery, University of California Irvine, Orange, <sup>4</sup>Depts. Surgery and Biomedical Engineering, University of California Irvine, Orange, USA.

**Background and aims:** Insulin (INS) replacement therapy using transplantation of pancreatic islets has held promise since reports of the Edmonton Protocol in diabetic patients. Efforts to improve islet viability and long-term functionality without chronic immunosuppression have spawned novel strategies including encapsulating islets in biocompatible polymers.

**Materials and methods:** Islet encapsulation into flat alginate sheets enables physiologic INS secretion from retrievable grafts. Islet viability and functionality was tested in vitro before or after s.c. transplantation into Lewis rats. Human islets from cadaveric donors from University of California Irvine were encapsulated into alginate hydrogel sheets after collagenase dissociation. Islet viability was quantified using Newport Green/propidium iodide. Islet function was determined from static glucose-stimulated INS release (GSIR), expressed as the Stimulation Index (SI; ratio of INS released at 28 mM and 2.8 mM glucose).

**Results:** Encapsulated human islets were  $95 \pm 0.2\%$  viable after encapsulation and 1 week in culture, and remained viable 8 weeks post-transplantation ( $73.0 \pm 1.5\%$ ). Glucose responsiveness (= SI) was  $3.8 \pm 0.2$  in tissue culture and  $3.0 \pm 0.5$ ,  $3.0 \pm 0.4$ ,  $1.5 \pm 0.5$ , and  $1.5 \pm 0.3$  at 1, 2, 4, and 8 weeks post-transplant, respectively. These results prompted further study to assess in vivo metabolic efficacy of alginate-encapsulated islets in a large animal model. INS-deficient diabetes will be induced in male dogs (BW = 25–30 kg) by surgical pancreatectomy (PANX). Glucose control will be maintained with rapid- and intermediate-acting INS (twice daily). Diabetes will be verified by absence of measurable endogenous INS and C-peptide response to i.v. glucose bolus (0.3 g/kg, IVGTT). To formulate islet sheets for canine transplantation, pancreata will be obtained from donor dogs after IVGTTs to verify robust  $\beta$ -cell function, using two donors per recipient to ensure adequate islet number for encapsulation. Two weeks post-PANX, diabetic dogs will be implanted with

four islet sheets into two ventral s.c. pockets. Functionality will be quantified from INS and C-peptide release during IVGTTs at day 5, 12, 26, and 54 post-transplantation, and repeated after graft explantation. *In vitro* islet viability and function will be tested by GSIR, and peri-sheet vascularization and lymphocyte infiltration will be assessed by histology of retrieved explant. **Conclusion:** Pancreatic islets encapsulated into alginate sheets demonstrate consistent viability and functionality in either tissue culture and within a small animal model, and are primed for comprehensive evaluation in a large animal model of INS-deficient diabetes. Canine studies described herein are critical for progression of this novel therapeutic modality towards clinical application.

*Supported by: Hanuman Medical Foundation*

## 440

### Preculture of islets with mesenchymal stem cells enhances islet function *in vitro* and produces superior transplantation outcome in diabetic mice

C.L. Rackham, P.K. Dhabda, A.A. Dattani, J.E. Bowe, P.M. Jones, A.J.F. King; Diabetes and Nutritional Sciences, King's College London, UK.

**Background and aims:** We have previously shown that co-transplanting mesenchymal stem cells (MSCs) enhances islet graft function and improves revascularisation *in vivo*. The aims of the present study were to determine whether preculturing islets with MSCs would improve islet function and maintain islet endothelial cell number *in vitro*. We also investigated whether preculturing islets with MSCs improves islet transplantation outcome in diabetic mice.

**Materials and methods:** Kidney MSCs and pancreatic islets were isolated from C57BL/6 mice. *In vitro*: Islets were cultured alone or with MSCs for three days using a direct contact co-culture system, after which islet insulin content and glucose-induced insulin secretion was measured. Islet beta cell number and endothelial cell number was analysed by histology. *In vivo*: Streptozotocin-diabetic C57BL/6 mice were implanted under the kidney capsule with a minimal mass of 150 islets which had been precultured alone or with MSCs. Blood glucose concentrations were monitored for 28 days. All cured mice were given an intraperitoneal glucose tolerance test at one month. Two days later the islet graft-bearing kidney was removed by nephrectomy to assess whether graft removal would result in reversion to hyperglycaemia.

**Results:** *In vitro*: Preculture of islets with MSCs potentiated glucose-stimulated (20mmol/l) insulin secretion ( $1.7 \pm 0.2$  vs  $2.8 \pm 0.3$  ng/islet/hr,  $p < 0.05$ ,  $n=5$ ). Islet insulin content was reduced in three day cultured islets, compared to those that were freshly isolated ( $39.8 \pm 3.6$  vs  $87.7 \pm 12.8$  ng/islet,  $p < 0.05$ ,  $n=5$ ). Preculturing islets with MSCs helped to prevent the decline in insulin content ( $77.4 \pm 6.3$  vs  $34.8 \pm 3.7$  ng/islet,  $p < 0.05$ ,  $n=5$ ). The number of insulin-positive beta cells per islet section was higher in MSC precultured islets compared to those cultured alone ( $83.7 \pm 5.1$  and  $54.4 \pm 6.5$  beta cells per islet section,  $n=4$ ,  $p < 0.05$ ). There was a rapid and dramatic loss of intraislet endothelial cells during culture ( $961.2 \pm 104.7$  vs  $72.9 \pm 9.0$  endothelial cells/mm<sup>2</sup>, freshly isolated vs. three day cultured islets,  $n=4$ ,  $p < 0.0001$ ). Preculturing islets with MSCs did not maintain intraislet endothelial cells ( $56.2 \pm 11.5$  vs  $48.8 \pm 12.4$  endothelial cells/mm<sup>2</sup>, MSC precultured islets and islets cultured alone,  $n=4$ ,  $p > 0.2$ ). *In vivo*: Islets which had been precultured with MSCs significantly lowered blood glucose at 7, 14, 21 and 28 days post-transplantation ( $p < 0.01$ ,  $n=10$ ). Day 28: Islet + MSC =  $8.2 \pm 0.4$  mmol/l vs islet alone =  $19.5 \pm 3.1$  mmol/l. At one month, all mice in the MSC precultured islets transplant group had cured compared to only 40% of mice transplanted with islets cultured alone. The glucose tolerance of cured mice was similar between transplant groups. All cured mice reverted to hyperglycaemia ( $>20$  mmol/l) on removal of the graft-bearing kidney. The density of endothelial cells was similar in the endocrine tissue of islet grafts consisting of islets cultured alone and islets precultured with MSCs ( $676 \pm 32.3$  vs  $631.8 \pm 80.2$  endothelial cells/mm<sup>2</sup>,  $n=4$ ,  $p > 0.05$ ).

**Conclusion:** Preculture of islets with MSCs does not affect endothelial cell number *in vitro* or *in vivo*, but does improve islet function and transplantation outcome. This may have implications for improving islet culture for clinical transplantation.

*Supported by: DUK, BBSRC, BPSIP, RCUK*

## 441

### The development of a method to preserve endothelium cells during human islet isolation

G.C. Huang<sup>1</sup>, M. Zhao<sup>1</sup>, P. Srinivasan<sup>2</sup>, P. Chondhary<sup>1</sup>, H. Tang<sup>1</sup>, N. Heaton<sup>2</sup>, S. Amiel<sup>1</sup>;

<sup>1</sup>Diabetes and Endocrinology, King's College London, <sup>2</sup>Liver Transplantation, King's College Hospital, London, UK.

**Background and aims:** Successful human islet transplantation depends not only on the mass of human islets and their viability but on how quickly the islets can get revascularisation. The current human islet isolation procedures deprive the endothelium tubelets from the islets. We aim to preserve more islets endothelium cells during the islet isolation to promote islet revascularisation following transplantation.

**Materials and methods:** Human pancreas organs with research consent were used in this study. Our modified method is to deliver collagenases and neutral protease (Serva) to pancreas in a sequential manner in comparison with those that the two types of enzyme were delivered at the same time (standard method). The enzymes and reagents used for the islet isolation is the same between the two methods. The silicon nitride marbles were removed from the Recordi's digestion chamber to avoid potential physical damages to islets. The pancreatic digestive was incubated with UWS supplemented with 2% of human albumin during tissue collecting phase. The ability of the islets to revascularise was assessed by transplanting the human islets under the kidney capsule in chemical induced diabetes mice. The graft-bearing kidneys were analysed by immunohistochemical staining.

**Results:** There were no obvious differences in the donor characteristics used in the study between the two methods. The means islet IEQ using modified method ( $n=21$ ) is  $390121 \pm 130015$  and their viability is  $85 \pm 6\%$ . The means islet IEQ using standard method ( $n=10$ ) is  $357321 \pm 113561$  and their viability is  $83 \pm 7\%$ . The islet yield and viability of the modified method are not significant difference from the standard method ( $P=0.08$  for the islet yield and  $p=0.15$  for the viability respectively). The islets from the modifying method, however, have  $20 \pm 10\%$  islets with attached endothelium cell tubelets in comparison with no endothelium cells attached to islets in the standard method. More interestingly these islets with endothelium cells attached seemed to have achieved rapid revascularisation within 3 days post transplant in diabetic mice, while the islets from standard methods showed little revascularisation on day 3 post transplantation.

**Conclusion:** Our modified method is a more gentle approach to generate human islets. This leads to the retaining of some endothelium cells with the islets. The retaining endothelium seemed to contribute the revascularisation processing and to improve the engraftment of islets. Islets from this modified method have higher capacity to revascularise and might have important implication in human islet transplantation.

*Supported by: King's College Hospital Charitable Trust*

## 442

### Resolving complexity of the IC2 autoantibody recognised target on the surface of functional beta cells by cellular and molecular affinity measurements

I.D. Pedersen<sup>1</sup>, K.L. Jensen<sup>1</sup>, C. Käck<sup>2</sup>, T. Aastrup<sup>2</sup>, M. Cárdenas<sup>3</sup>, M. Blaise<sup>4</sup>, K. Buschard<sup>1</sup>, C.-H. Brogren<sup>1</sup>;

<sup>1</sup>The Bartholin Institute, Rigshospitalet, Copenhagen, Denmark, <sup>2</sup>Attana AB, Stockholm, Sweden, <sup>3</sup>Nano-Science Center, University of Copenhagen, Denmark, <sup>4</sup>Department of Molecular Biology, Aarhus University, Denmark.

**Background and aims:** The rat monoclonal autoantibody IC2, raised in a newly diabetic BB-rat, has been shown to have a unique specificity for the surface of insulin secreting pancreatic  $\beta$ -cells. The target autoantigen for IC2 has been partially characterized by high-performance thin-layer chromatography combined with radioimmunoassay and lipid microarrays and it appears to be specific for sulphated glycolipids and sphingomyelins isolated from RIN-5F or INS-1E rat  $\beta$ -cells as well as their purified plasma membranes. Previous affinity measurements towards  $\beta$ -cells, sulfatides and sphingomyelins indicate a complex nature of the IC2 autoantigen. The reactivity seems to be directed primarily towards galactose-3-sulphate on sulfatides and phosphocholine residues on sphingomyelins though also phosphatidylcholines exhibits binding. Trypsin treatment abolishes IC2 binding to  $\beta$ -cells, which indicates that a protein structure might either be a part of the  $\beta$ -cell surface autoantigen, or function as carrier for the autoantigen. Recently, IC2 was found to have an inhibitory effect on NKT type I and II cells which points

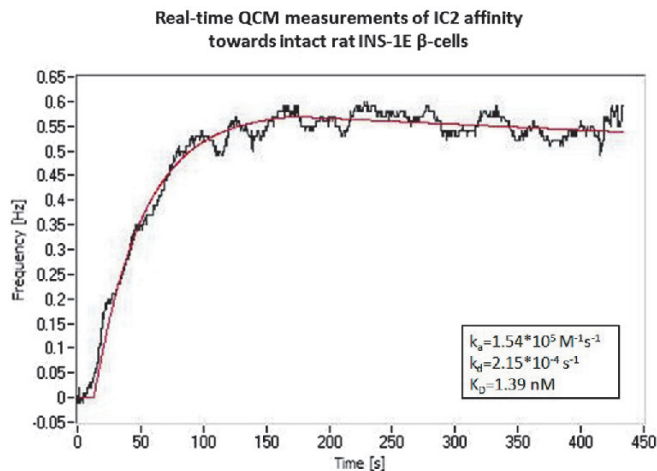


towards formation of the targeted epitope in a complex between the pancreatic islet  $\beta$ -cell expressed CD1d and noncovalently anchored phospho- and glycolipids, since these are known to stimulate NKT cells.

**Materials and methods:** Both cellular and molecular real-time quartz crystal microbalance (QCM) experiments and molecular microscale thermophoresis (MST) experiments were performed. Affinities were measured towards intact INS-1E cells, sonicated plasma membrane vesicles, lipid rafts, lipids, and lipids anchored to the CD1d protein, as well as several different complexes and combinations of these.

**Results:** Cellular QCM showed that IC2 has a very strong affinity towards intact INS-1E cells with a dissociation constant of 1.39 nM and an association rate of  $1.54 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$  (Figure). Several different molecular components were examined by molecular QCM. MST measurements showed a medium-strong affinity of IC2 towards lysosulfatide with a dissociation constant of 1  $\mu\text{M}$ . Affinities were compared and ranked in order to elucidate and resolve the complexity of the autoantigen.

**Conclusion:** Getting closer to a characterization of the autoantigen leads to potential new therapeutic approaches by using IC2 in drug delivery. These results are also of massive importance to the development of IC2 as a functional biomarker for the  $\beta$ -cell mass. This could lead to a characterization of the diabetic process and an improved focus of therapeutic interventions in both T1DM and T2DM patients, by being able to closely follow the effects of treatment with growth promoting drugs (e.g. GLP-1 agonists), stem-cell therapy, and islet transplantation.



Supported by: JDRF International and EU-Fasilis Program

## PS 021 Experimental immunology and models of type 1 diabetes

443

### Evaluation of immunogenicity of insulin-producing gut cells in diabetes-prone mice

M. Mojibian<sup>1</sup>, A.W.Y. Lam<sup>1</sup>, Y. Fujita<sup>2</sup>, A. Asadi<sup>1</sup>, G.A. Grassl<sup>3</sup>, P. Dickie<sup>4</sup>, A.T. Cheung<sup>5</sup>, T.J. Kieffer<sup>1</sup>;

<sup>1</sup>Cellular & Physiological Sciences, University of British Columbia, Vancouver, Canada, <sup>2</sup>Department of Internal Medicine, Asahikawa Medical University, Asahikawa, Hokkaido, Japan, <sup>3</sup>Michael Smith Laboratories, Vancouver, Canada, <sup>4</sup>University of Alberta, Edmonton, Canada, <sup>5</sup>enGene, Inc., Vancouver, Canada.

**Background and aims:** Type 1 diabetes is caused by autoimmune destruction of pancreatic  $\beta$ -cells, but the mechanisms involved are not well-understood. Intestinal K-cells are glucose-responsive, native endocrine cells that may be good candidates for engineering into surrogate  $\beta$ -cells to restore endogenous insulin secretion. We have generated diabetes-prone NOD (non-obese diabetic) transgenic mice with targeted expression of insulin to gut K-cells (GIP/Ins mice). These transgenic mice are protected from developing both chemically-induced and spontaneous diabetes. The aim of this study was to determine the immunogenicity and impact of insulin producing gut K-cells in NOD mice.

**Material and methods:** The incidence of spontaneous diabetes was determined in transgenic and non-transgenic NOD mice. Histology and insulin immunoreactivity were evaluated in pancreas and duodenum of these animals. We evaluated T cell immune responses to insulin using a CFSE proliferation assay and the frequency of circulating T cells with a tetramer assay. Serial plasma samples were collected from 6-24 weeks to profile insulin autoantibodies.

**Results:** The incidence of hyperglycemia was markedly reduced in 2 cohorts of GIP/Ins NOD mice that produced insulin in K-cells (non-transgenic vs transgenic: 100% (n=22) vs 45% (n=19) at 32 wks, and 90% (n=11) vs 15% (n=14) at 44 wks).  $\beta$ -cell inflammation and damage were reduced in pancreas of transgenic mice compared to controls, and insulin<sup>+</sup> K-cells remained intact with no sign of inflammation. We evaluated pancreas sections from 32 wk transgenic mice, an age by which all non-transgenic mice had succumbed to diabetes (n=22), and found that 7 of 10 transgenic mice had little or no insulinitis. In a different cohort of 32 wk old mice, we examined the proliferation of cells isolated from mesenteric lymph nodes (MLN) in the presence of insulin; the cell division index in MLN cells from transgenic mice was significantly lower compared to non-transgenic mice ( $p < 0.03$ ). At 15 wks (before diabetes) transgenic animals had a lower frequency of peripheral CD8<sup>+</sup> T cells specific to NRP-V7 compared with controls ( $p < 0.03$ ). We also detected a reduced number of insulin autoantibody-positive transgenic animals compared to controls (cumulative incidence of 46 vs 80% in non-transgenic vs transgenic mice, respectively by 24 wks). Moreover, while there was a clear predictive value in the number of insulin autoantibody-positive control mice that subsequently developed diabetes (88%), this was not the case in the GIP/Ins transgenic mice (16%).

**Conclusion:** Insulin is generally thought to be a key autoantigen responsible for the selective elimination of  $\beta$ -cells. However, our data indicate that gut-derived insulin not only prevents hyperglycemia, but also provides protection against diabetes in the setting of autoimmune assault against  $\beta$ -cells. Collectively, our results suggest that gut-derived insulin reduces the immune response to insulin, which may explain the protection of gut insulin-producing cells, and also the delay in immune attack of pancreatic  $\beta$ -cells. Targeting gut K-cells may be useful for insulin replacement therapy, as well as inducing tolerance and diminishing autoimmunity.

Supported by: JDRF

## 444

**Pancreatic overexpression of IGF-I protects NOD mice from autoimmune diabetes**C. Mallol<sup>1,2</sup>, A. Casellas<sup>1,2</sup>, V. Jimenez<sup>1,2</sup>, E. Casaña<sup>1,2</sup>, M. Morro<sup>1,2</sup>, M. Obach<sup>1,2</sup>, J. Agudo<sup>1,2</sup>, E. Ayuso<sup>1,2</sup>, F. Bosch<sup>1,2</sup>;<sup>1</sup>Department of Biochemistry and Molecular Biology and Centre of Animal Biotechnology and Gene Therapy, Universitat Autònoma de Barcelona, Bellaterra, <sup>2</sup>CIBERDEM, Barcelona, Spain.

**Background and aims:** Both type 1 and 2 diabetes result from reduced  $\beta$ -cell mass. Recent studies have implicated insulin and insulin-like growth factor I (IGF-I) signaling pathways in the regulation of  $\beta$ -cell proliferation. We have previously shown that expression of IGF-I under control of the rat insulin-I promoter (RIP-I) in  $\beta$ -cells of transgenic mice counteracts cytotoxicity and insulinitis, and regenerates the endocrine pancreas after treatment with streptozotocin. In addition,  $\beta$ -cell expression of IGF-I protects islets from autoimmune destruction in IFN- $\beta$  transgenic mice, a model of lymphocytic infiltration of endocrine pancreas. To further investigate the role of IGF-I preventing from autoimmune diabetes, here we analyze the effect of IGF-I expression in  $\beta$ -cells of type 1 diabetes-prone Nonobese Diabetic (NOD) mice.

**Materials and methods:** Transgenic mice overexpressing murine IGF-I under the control of the RIP-I Promoter were backcrossed with NOD mice for 15 generations to obtain IGF-I transgenic mice with 99% NOD genetic background. Blood glucose levels and other metabolic parameters, body weight, and leukocyte infiltration were analyzed.

**Results:** Pancreatic expression of IGF-I in  $\beta$ -cells of NOD transgenic mice prevented the development of diabetes. While 63% of wild type NOD mice developed diabetes at 30 weeks of age, only 10% of NOD IGF-I transgenic mice became diabetic, and 80% of individuals remained normoglycemic at the age of 10 months. Thus, the incidence of autoimmune diabetes in female NOD IGF-I transgenic mice was significantly reduced. Immunostaining against insulin revealed a preservation of  $\beta$ -cell mass and a reduction of leukocytic infiltration of islets. Serum insulin levels in NOD transgenic mice were also higher than in control mice. To further confirm the protective effect of IGF-I, transgenic RIP/IFN $\beta$  mice were injected via retrograde pancreatic intraductal administration with adeno-associated viral vectors (AAV) expressing IGF-I under control of the RIP promoter. Preliminary results indicate that AAV-mediated expression of IGF-I into the pancreas of adult RIP/IFN $\beta$  mice is also able to prevent the diabetic process. Studies investigating the molecular mechanisms by which IGF-I exerts this protection are being conducted in our laboratory.

**Conclusion:** This study indicates that local expression of IGF-I in the pancreas prevents islet infiltration and  $\beta$ -cell death in mice with increased susceptibility to type 1 diabetes development. Thus, IGF-I may represent a key factor to prevent autoimmune destruction of  $\beta$ -cells in type 1 diabetes.

**Supported by:** Ministerio de Ciencia e Innovación, Plan Nacional I+D+I (SAF2008-00962)

## 445

**Toll-like receptor 4 deficiency mediates insulin resistance in prediabetic NOD mice and accelerates the progression of autoimmune-mediated diabetes**A.L. Reinbeck<sup>1</sup>, G. Séquaris<sup>1</sup>, H.J. Partke<sup>1</sup>, P. Nowotny<sup>1</sup>, J. Kotzka<sup>2</sup>, B. Knebel<sup>2</sup>, V. Burkart<sup>1</sup>, M. Roden<sup>1,3</sup>;<sup>1</sup>Institute for Clinical Diabetology, German Diabetes Center, Duesseldorf,<sup>2</sup>Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Duesseldorf, <sup>3</sup>Department of Metabolic Diseases, University Clinics Duesseldorf, Germany.

**Background and aims:** The pathogenesis of type 1 diabetes is characterized by immune-mediated destruction of autologous pancreatic beta cells. Recent observations indicate that disturbances of energy metabolism contribute to disease progression. Previous studies demonstrated an important role of toll-like receptor 4, a receptor for lipopolysaccharide on innate immune cells, in the control of (auto-)immune processes as well as energy metabolism. We therefore hypothesized that TLR4 determines a diabetes-modulating metabolic phenotype in non-obese diabetic (NOD) mice.

**Materials and methods:** Age and blood glucose levels at diabetes manifestation were monitored in female TLR4-expressing (TLR4<sup>+/+</sup>) and TLR4-deficient (TLR4<sup>-/-</sup>) NOD mice. Glucose tolerance was assessed in 6-hours fasted mice by intraperitoneal glucose tolerance tests (ipGTT). Mitochondrial func-

tion was determined by high-resolution respirometry. Insulin and IL-6 serum concentrations were quantified by sandwich ELISA.

**Results:** TLR4<sup>-/-</sup> NOD mice developed diabetes earlier in life ( $152 \pm 25$  days) than TLR4<sup>+/+</sup> NOD mice ( $208 \pm 40$  days,  $p < 0.001$ ). Furthermore, TLR4<sup>-/-</sup> NOD mice had increased blood glucose concentrations at the onset of diabetes when compared to TLR4<sup>+/+</sup> mice (TLR4<sup>-/-</sup>  $457 \pm 30$ , TLR4<sup>+/+</sup>  $362 \pm 44$  mg/dl,  $p < 0.001$ ). Moreover, in the prediabetic period, normoglycemic TLR4<sup>-/-</sup> mice had markedly lower glucose tolerance (area under the glucose curve: TLR4<sup>-/-</sup>  $587 \pm 152$  mmol/L x h<sup>-1</sup>, TLR4<sup>+/+</sup>  $233 \pm 102$  mmol/L x h<sup>-1</sup>,  $p < 0.001$ ). Upon ipGTT, insulin concentrations were doubled in TLR4<sup>-/-</sup> compared with TLR4<sup>+/+</sup> mice. Fasting serum free fatty acid levels were 40% higher in TLR4<sup>-/-</sup> than in TLR4<sup>+/+</sup> mice. In TLR4<sup>-/-</sup> NOD mice, hepatic but not myocellular mitochondria showed increased O<sub>2</sub>-flux through complex I and II (TLR4<sup>-/-</sup>  $54.6 \pm 14.8$ , TLR4<sup>+/+</sup>  $29.6 \pm 7.4$  (pmol x mg<sup>-1</sup> x s<sup>-1</sup>)/mtDNA copy number,  $p < 0.05$ ) and higher maximal respiratory capacity (TLR4<sup>-/-</sup>  $87.8 \pm 18.6$ , TLR4<sup>+/+</sup>  $55.5 \pm 22.7$  (pmol x mg<sup>-1</sup> x s<sup>-1</sup>)/mtDNA copy number,  $p < 0.01$ ) when compared to TLR4<sup>+/+</sup> NOD mice. Furthermore, TLR4-deficient NOD mice had higher serum concentrations of the proinflammatory cytokine interleukin-6 (TLR4<sup>-/-</sup>  $4.8 \pm 0.9$  pg/ml, TLR4<sup>+/+</sup>  $2.3 \pm 0.5$  pg/ml;  $p < 0.001$ ).

**Conclusion:** Taken together, TLR4 deficiency decreases insulin sensitivity resulting in increased substrate availability and subsequently enhanced O<sub>2</sub>-flux in liver mitochondria. This indicates that TLR4 controls the progression of immune-mediated diabetes in NOD mice by impairing insulin sensitivity.

## 446

**The pathology underlying beta cell destruction in four type 1 diabetes animal models in comparison with the human situation**A. Jörns<sup>1</sup>, C. Mathieu<sup>2</sup>, C. Gysemans<sup>2</sup>, F. Scott<sup>3</sup>, G.-S. Wang<sup>3</sup>, Y. Nakaya<sup>4</sup>, N. Harada<sup>4</sup>, P. Marchetti<sup>5</sup>, L. Marselli<sup>5</sup>, S. Lenzen<sup>1</sup>;<sup>1</sup>Institute of Clinical Biochemistry, Hannover Medical School, Germany,<sup>2</sup>Laboratory for Experimental Medicine and Endocrinology, Catholic University of Leuven, Belgium, <sup>3</sup>Chronic Disease Program, Ottawa Hospital Research Institute, Canada, <sup>4</sup>Institute of Health Biosciences, University of Tokushima Graduate School, Japan, <sup>5</sup>Section of Endocrinology and Metabolism of Organ Transplantation, University of Pisa, Italy.

**Background and aims:** The aim of the study was to compare the immune cell infiltrate and the cytokine pattern in pancreatic islets leading to beta cell loss in the four spontaneous type 1 diabetes animal models, namely the NOD mouse, the BB rat, the LEW.1AR1-*iddm* rat, and the Komedra rat with the human situation after type 1 diabetes development.

**Materials and methods:** After diabetes manifestation pancreases from NOD mice, BB, LEW.1AR1-*iddm*, and Komedra rats were analysed by immunohistochemistry and by *in situ* RT-PCR for the immune cell composition of the infiltrate and the cytokine pattern including proliferation and apoptosis rate in comparison with the human situation and normoglycaemic controls.

**Results:** In the pancreatic islets from normoglycaemic controls of the rodents as well as of humans beta cells were the main cell type; there were no signs of immune cell infiltration both in the islets and in the exocrine pancreatic parenchyma including the vessels. Without infiltration no cytokine expression was observed in any pancreatic parenchymal cell type. After type 1 diabetes manifestation an immune cell infiltration was found in the islets. With declining frequency CD8 T cells, CD68 macrophages, and CD4 T cells were observed as the main immune cell types, independent from the analysed animal model as well as in pancreases from patients. In the human situation B cells and in the NOD mice regulatory T cells expressing FoxP3 were more frequently found. IL-1 $\beta$  and TNF- $\alpha$  were expressed as the main pro-inflammatory cytokines in the immune cell infiltrate in the NOD mice, BB and LEW.1AR1-*iddm* rats, and in the human situation while in the Komedra rat, as an exception, IFN- $\gamma$  and TNF- $\alpha$  were the main cytokines as a sign of immune cell activation. Additionally, the autoimmune process supporting IL-17 cytokine was expressed in infiltrated islets. Also, the anti-inflammatory cytokines, IL-4, IL-10, and IL-13 were found in some immune cells in each islet infiltrate. Ultrastructurally confirmed apoptotic beta cell death (apoptosis rate between 0.9 - 3.2 % by the TUNEL assay) was observed in the pancreatic islets from the animal models and also in the human situation. Under all diabetic conditions proliferating beta cells (proliferation rate between 0.5 - 2.1 % by the Ki67 and insulin immunostaining), were also found in islets with remaining beta cells.

**Conclusion:** The main immune cells, cytotoxic CD8 T cells, CD4 T cells, and CD68 macrophages, were similar in all animal models to the human situation. Pro-inflammatory patterns were TNF- $\alpha$ , IL-1 $\beta$ , and IFN- $\gamma$  with decreasing frequency expressed in the infiltrating immune cells as a sign of activation

leading to beta cell apoptosis. The exception was the Komeda rat, where a dominance of IFN- $\gamma$  instead of IL-1 $\beta$  expression was observed in parallel to TNF- $\alpha$  expression. Thus animal models of type 1 diabetes can serve as good models providing meaningful information also for the understanding of the disease processes in the pancreas of patients with type 1 diabetes.

## 447

### Treg-mediated suppression of autoimmune diabetes in mice following non-viral-mediated hepatic overexpression of IGF-I

F. Bosch<sup>1</sup>, S. Tafuro<sup>2,3</sup>, C. Roca<sup>2,3</sup>, D. Callejas<sup>2,3</sup>, J. Agudo<sup>2,3</sup>, M. Obach<sup>2,3</sup>, A. Ruzo<sup>2,3</sup>, C.J. Mann<sup>2,3</sup>, A. Casellas<sup>2,3</sup>, X.M. Anguela<sup>2,3</sup>;  
<sup>1</sup>CIBERDEM, Universitat Autònoma de Barcelona, Bellaterra, <sup>2</sup>Department of Biochemistry and Molecular Biology, School of Veterinary Medicine, Universitat Autònoma de Barcelona, Bellaterra, <sup>3</sup>CIBERDEM, Barcelona, Spain.

**Background and aims:** In type 1 diabetes, loss of tolerance to  $\beta$ -cell antigens results in T cell-dependent autoimmune destruction of  $\beta$ -cells. The abrogation of autoreactive T cell responses is a prerequisite to achieve long-lasting correction of the disease. Hepatic gene transfer may result in tolerance induction and suppression of autoimmune diseases, in part by regulatory T cell (Treg) activation. The unique immunomodulatory properties of the liver could hence be manipulated to treat or prevent diabetes onset through expression of key genes. Thus, in the context of an autoimmune disease like type 1 diabetes, a liver-targeted gene therapy approach to modify peripheral control of autoreactive lymphocytes offers a unique possibility to prevent development and progression of the disease. Insulin-like growth factor-I (IGF-I) may be an immunomodulatory candidate since it prevents autoimmune diabetes when expressed in  $\beta$ -cells or subcutaneously injected. We have previously reported that local expression of IGF-I also protects islets from autoimmune destruction in an IFN- $\beta$  transgenic mouse model of type 1 diabetes. All these studies reveal IGF-I as a potential key factor to induce protection from autoimmune diabetes. Here, we aimed to determine whether transient IGF-I overexpression in mouse liver may suppress autoimmune diabetes progression.

**Materials and methods:** Heterozygous male transgenic mice expressing human IFN $\beta$  under the control of the rat insulin promoter-I (RIP-I) treated with streptozotocin (25 mg/kg bw) were used as a model of type 1 diabetes. Hepatic expression of IGF-I and eGFP was achieved following hydrodynamic tail vein injection via the lateral vein of plasmid DNA in saline in a volume equal to ~10% of body weight in less than 5 seconds. eGFP and IGF-I were expressed from plasmids containing either the CAG, hAAT or CD68 promoters. **Results:** In this work, we report a decrease in the incidence of diabetes following hepatic delivery of a plasmid expressing IGF-I by hydrodynamic tail vein (HTV) injection, in an autoimmune mouse model of the disease. We show that the expression of IGF-I in liver non-parenchymal cells leads to reduced  $\beta$ -cell apoptosis, increased  $\beta$ -cell replication and decreased pancreatic infiltration by immune effector cells, suggesting a blockage of the autoimmune attack against the pancreas. As a consequence, normalized  $\beta$ -cell mass, circulating insulin levels and glucose tolerance in STZ-treated mice were achieved. Permanent protection depended on IGF-I expression in liver non-parenchymal cells and was associated with increased percentage of intrahepatic and intrapancreatic Tregs. Importantly, Treg depletion completely abolished IGF-I-mediated protection confirming the therapeutic potential of these cells in autoimmune diabetes.

**Conclusion:** This study demonstrates that a non-viral gene therapy combining the immunological properties of the liver and IGF-I could be beneficial in the treatment of the disease.

Supported by: Ministerio de Ciencia e Innovación (SAF2008-00962)

## 448

### Overexpression of vitamin-D receptor in beta cells alters beta cell functionality but protects mice from diabetes

A. Casellas<sup>1,2</sup>, C. Mallol<sup>1,2</sup>, V. Jimenez<sup>1,2</sup>, M. Obach<sup>1,2</sup>, M. Morro<sup>1,2</sup>, J. Agudo<sup>1,2</sup>, E. Ayuso<sup>1,2</sup>, M. Molas<sup>1,2</sup>, R. Lage<sup>1,2</sup>, F. Bosch<sup>1,2</sup>;  
<sup>1</sup>Department of Biochemistry and Molecular Biology and Center of Biotechnology and Gene Therapy, Universitat Autònoma de Barcelona, Bellaterra, <sup>2</sup>CIBERDEM, Barcelona, Spain.

**Background and aims:** Vitamin D3 is obtained through the diet or by synthesis in skin, but only the 1,25(OH)<sub>2</sub>D3 form of vitamin D is metabolically active. This molecule exerts its effects by activating the nuclear vitamin D

receptor (VDR), which is a member of the nuclear receptor superfamily. Epidemiological studies have revealed an association between VDR gene polymorphism and diabetes. It has also been shown that vitamin D deficiency predisposes individuals to diabetes, and inhibits pancreatic insulin secretion in rodents and rabbits. Here, we aimed to determine the role of VDR in  $\beta$ -cell physiology and its effects in diabetes protection.

**Materials and methods:** Transgenic mice overexpressing murine VDR specifically in  $\beta$ -cells under control of the Rat Insulin Promoter-I (RIP-I) were obtained. Blood glucose levels, body weight and other metabolic parameters were analyzed and glucose tolerance test were performed. To induce diabetes, two-month-old mice were treated with low doses of streptozotocin (STZ, 5x50 mg/ Kg bw).

**Results:** Three lines (L1-L3) of RIP/VDR transgenic mice were obtained. All lines expressed VDR in islets. After double immunostaining against insulin and VDR we observed that the VDR expression was restricted to  $\beta$ -cells. The VDR-L1 line, which presented the highest level of expression of the transgene, showed slight hyperglycemia compared to wild type littermates in fed and fasted conditions, although insulin levels were not significantly different between the two groups. Moreover, VDR-L1 transgenic mice showed an increase in  $\beta$ -cell mass, and impaired glucose tolerance. VDR-L3, which showed low expression of the transgene, also exhibited impaired glucose tolerance, but no alteration in fed and fasting glycemia. To examine the role of VDR in  $\beta$ -cell protection against damage, wild type and VDR-L1 transgenic mice were treated with STZ. In contrast to STZ-treated wild type mice, which were hyperglycemic, STZ-treated VDR-L1 transgenic mice showed a reduction in glycemia and improved glucose tolerance test. These results correlated with serum insulin levels, which were higher in STZ-treated transgenic mice than in STZ-treated wild type mice.  $\beta$ -cell mass was also higher in STZ-treated VDR-L1 transgenic mice. Further studies aimed at understand the effects of VDR overexpression in  $\beta$  cells are presently carried out in our laboratory.

**Conclusion:** These results indicate that local overexpression of VDR in  $\beta$ -cells affects  $\beta$  cell functionality. Moreover, VDR overexpression may protect mice from diabetes.

Supported by: Grants from Ministerio de Ciencia e Innovación

## 449

### Prediction and prevention of islet infiltration using RIB5/2 antibody in the LEW.1AR1-iddm rat model of autoimmune diabetes

T. Schoeppe<sup>1</sup>, H. Weiss<sup>1</sup>, G. Fuellen<sup>2</sup>, M. Tiedge<sup>1</sup>;  
<sup>1</sup>IBIO, University of Rostock, <sup>2</sup>IBIMA, University of Rostock, Germany.

**Background and aims:** The LEW.1AR1-iddm rat is an animal model of spontaneous autoimmune diabetes. Islet infiltration occurs within a narrow time range resulting in progressive beta cell destruction and overt diabetes. It was the aim of this study to investigate (1) the predictive potential of gene expression profiling in peripheral mononuclear cells of the blood (PBMC) and (2) tolerance-inducing effects through treatment by the modulating anti-CD4 antibody RIB5/2 on islet infiltration in the prediabetic period.

**Materials and methods:** 6 LEW.1AR1-iddm rats were treated at the age of 40, 42, 44, 46 and 48 days with the monoclonal antibody RIB5/2 (5 x 5 mg Ab/kg b.w. i.p.). 200  $\mu$ l blood were collected at day 40 before antibody application and day 60. Normoglycaemic LEW.1AR1-iddm rats were killed at day 40 (n = 16). Serial pancreatic sections were stained with Haematoxylin-Eosin (HE) to document the status of infiltration. RNA was isolated from gradient-purified PBMCs. Gene expression was quantified for proinflammatory (TNF $\alpha$ , IFN $\gamma$ , IL-1 $\beta$ ) and antiinflammatory cytokines (IL-4, IL-10), T-cell markers (CD25, CTLA4, NRP1), L-Selectin, TGF- $\beta$  and FoxP3 by RT-PCR. The RT-PCR results were analysed by decision tree classification in the weka data mining tool using C4.5 algorithm for day 40.

**Results:** The decision tree classification showed, that from our panel of analysed genes, IL-1 $\beta$  expression (IL-1 $\beta$ /GAPDH ratio >144.66) was predictive in PBMCs for pancreatic infiltrations at day 40. Treatment with the anti-CD4 antibody RIB5/2 resulted in a significantly (P<0.05) reduced gene expression levels of IL-1 $\beta$ , TNF $\alpha$ , IL-10, NRP1 and TGF- $\beta$  (1.4 - 2.3-fold) 12 days after the last administration of RIB5/2 (d60). The reduction of IL-1 $\beta$  expression due to RIB treatment was able to prevent 100 % of the animals from infiltrations. At day 60 RIB5/2 treated animals showed a significant (P<0.05) reduction for PBMC gene expression of L-Selectin (4.5-fold), CD25 (2.6-fold), TGF- $\beta$  (2-fold), FoxP3 (2.7-fold) and CTLA4 (16-fold) compared with non-infiltrated rats. Gene expression levels of IL-4 was significantly higher (3.6-fold, P<0.05) in non-infiltrated animals. Those animals showing pancreatic infiltrations revealed significant (P<0.05) higher gene expression profiles of the cytokines TNF $\alpha$  (4.5-fold), IFN $\gamma$  (3.6-fold)



compared to RIB ab treated animals at day 60. Also the T-cell marker gene NRP1 (8-fold) was upregulated at this late point of autoimmunity. We also found that a significant ( $P<0.05$ ) reduction of the regulatory marker FoxP3 (2.3-fold) and the T-cell ligand L-Selectin (4.9-fold) in infiltrated animals was linked to a 7.5-fold reduced CTLA4 expression (T-cell silencing). **Conclusion:** Decision tree analyses of PBMCs allow to predict different stages of islet infiltrations of normoglycaemic LEW.1AR1-*iddm* rats. The anti-CD4 antibody RIB5/2 could induce tolerance against beta cell autoimmunity that induced gene signatures in PBMCs. IL-1 $\beta$  expression in combination with L-Selectin and TNF $\alpha$  proved to be a predictive marker for the early phase of islet infiltration. RIB resulted in a downregulation of proinflammatory cytokines genes and increased levels of regulatory T-cell marker genes. Our data indicate that analysis of gene expression profiles in PBMCs from risk patients could be an attractive strategy to monitor islet autoimmunity. Furthermore our data favor modulatory anti-CD4 abs as an attractive option for prevention of overt T1DM.

Supported by: EFSD/GSK grant

## 450

### Role of vitamin D in regulation of phagocytic function and CD4/CD8 splenic lymphocyte ratios in mice with STZ-induced diabetes

D. Labudzynski, I. Shymanskyi, V. Riasnyi, M. Veliky;

Laboratory of medicine biochemistry, Palladin Institute of Biochemistry, Kyiv, Ukraine.

**Background and aims:** Growing evidence suggests that alterations arising from abnormal immune responses are the major causes of type 1 diabetes and its complications. Beyond its classical calcium-regulating role in bone metabolism, vitamin D3 (D3) is currently recognized as a potent immunomodulator affecting the activities of macrophages, monocytes and lymphocytes in various autoimmune diseases. However, the precise mechanisms of D3 influence on immune homeostasis, immunity and tolerance in diabetes has not been clearly defined. We therefore investigated the relationship between D3 status and such immune parameters as functional competence of peripheral blood phagocytes and CD4/CD8 splenic T-lymphocyte ratios in diabetes and after chronic administration of D3.

**Materials and methods:** Type 1 diabetes was induced in male C57BL/6 mice (weighing  $25.0 \pm 1.5$ g) by i.p. injection of multiple low dose streptozotocin (40 mg/kg b.w.). Control and STZ-diabetic mice were maintained with or without treatment with D3, at 15 IU/mouse per os, for 8 weeks (a prevention paradigm). Serum 25-hydroxyvitamin D3 (25OHD3) was assessed by ELISA. The neutrophil and monocyte phagocytosis after exposure to FITC-labeled *E. coli* was determined quantitatively using flow cytometry and qualitatively by confocal laser scanning microscopy. Intracellular reactive oxygen species (ROS) were detected by 2',7'-dichlorofluorescein (DCF) fluorescence. Spleen lymphocytes were phenotyped using direct immunofluorescence staining after incubation with monoclonal antibodies specific for CD4 and CD8 cell markers by flow cytometry.

**Results:** Serum level of 25OHD3, the main circulating metabolite of D3, was shown to be reduced to  $23.8 \pm 1.9$  in diabetes vs.  $39.7 \pm 2.9$  in control, indicative of diabetes-induced D3 deficiency ( $p<0.05$ ). These changes were accompanied by decreased percentage of polymorphonuclear neutrophils and monocytes which ingest FITC-labeled *E. coli* and their activity (number of bacteria per cell) in diabetic mice. As further evidence of diabetes-associated dysfunction of peripheral blood phagocytes that may lead to impaired host defense against bacterial or fungal infections, decreased leukocytes ability to oxidize the fluorogenic substrate DCF was also found. In addition, lower immunoregulatory index indicating alteration of CD4/CD8 (helper/suppressor) lymphocyte ratio in spleen was observed in diabetic group as compared to control ( $1.43 \pm 0.12$  vs.  $1.80 \pm 0.15$  respectively,  $p<0.05$ ). Complete normalization of circulatory 25OHD3 levels was achieved due to D3 treatment. D3 administered to diabetic mice effectively restored production of ROS and phagocytic activity of cells towards the control values. Furthermore, it notably attenuated the immune imbalance observed in diabetes by up-regulating CD4/CD8 splenic lymphocyte ratio. D3 therapy had no effect on normal mice, except that the compound could augment the 25OHD3 levels. D3 treatment provided beneficial effects beyond glucose control because hyperglycaemia remained only slightly affected.

**Conclusion:** The study confirmed that diabetes was associated with impairment of phagocytic activity and redistribution in splenic lymphocyte subsets that correlated with vitamin D3 deficits. D3 possesses immunotherapeutic effects on mice with type 1 diabetes through improving the cell-mediated immunity.

## 451

### Disease recurrence after syngeneic islet transplantation in diabetic NOD mice is driven exclusively by pre-activated autoreactive T-cells

G.M. Alkemade<sup>1,2</sup>, X. Clemente-Casares<sup>1</sup>, J. Zhenguo Yu<sup>1</sup>, J. Wang<sup>1</sup>, B.-Y. Xu<sup>3,4</sup>, J.R. Wright, Jr.<sup>3</sup>, B.O. Roep<sup>2</sup>, P. Santamaria<sup>1,5</sup>;

<sup>1</sup>Julia McFarlane Diabetes Research Center, University of Calgary, Canada,

<sup>2</sup>Department of Immunohematology and Blood Transfusion, Leiden

University Medical Center, Netherlands, <sup>3</sup>Department of Pathology,

University of Calgary, Canada, <sup>4</sup>Alberta Diabetes Institute, University of

Alberta, Edmonton, Canada, <sup>5</sup>Institut d'Investigacions Biomèdiques August

Pi i Sunyer, Barcelona, Spain.

**Background and aims:** We have recently shown that T-cell recruitment to a site of autoimmune inflammation results from an active process that is strictly dependent on local display of cognate pMHC. We tested whether this revised paradigm also applies to recruitment of islet-specific T-cells into islets transplanted under the kidney capsule of diabetic NOD mice. Specifically, we addressed the contribution of memory IGRP<sup>206-214</sup> + CD8<sup>+</sup> T-cell in graft failure.

**Materials and methods:** Recruitment of IGRP<sup>206-214</sup> + CD8<sup>+</sup> cells into islet grafts from NOD.*scid* (expressing IGRP<sup>206-214</sup>) or NOD.*rag2*<sup>-/-</sup>.IGRP<sup>K209A/F213A</sup> donors (lacking IGRP<sup>206-214</sup>) was tracked in diabetic NOD hosts (harboring both naïve and memory IGRP<sup>206-214</sup> + CD8<sup>+</sup> cells) or diabetic NOD.IGRP<sup>K209A/F213A</sup> hosts (harboring naïve IGRP<sup>206-214</sup> + CD8<sup>+</sup> cells only) (n=5/ group). The presence of graft associated IGRP<sup>206-214</sup> + CD8<sup>+</sup> cells was quantitated through NRP-V7 tetramer staining and FACS analysis and IFN $\gamma$  secretion upon exposure to NRP-V7-pulsed dendritic cells was measured.

**Results:** Diabetes recurred significantly later in NOD hosts receiving IGRP-negative islet grafts than in those receiving wildtype islets (mean graft survival  $13.6 \pm 5.0$  vs  $5.5 \pm 0.7$  days;  $p<0.01$ ). IGRP<sup>206-214</sup> + T-cells were undetectable in IGRP-negative islet grafts, but accounted for a major fraction of T cells infiltrating IGRP<sup>+</sup> islet grafts ( $p=0.004$ ). In IGRP naïve hosts, IGRP<sup>206-214</sup> + T-cells remained undetectable in both IGRP+ islet grafts and islet grafts lacking this epitope.

**Conclusion:** Recognition of islet antigen is a prerequisite for T-cell recruitment in endogenous islets as well as in islet autografts. IGRP<sup>206-214</sup> + CD8<sup>+</sup> T-cells contribute to, but are dispensable for, rejection of islet grafts in diabetic NOD mice. Islets, transplanted under the kidney capsule, neither activate nor recruit naïve IGRP<sup>206-214</sup> -reactive CD8<sup>+</sup> T-cells early in the recurrent autoimmune response. Instead, recurrent diabetes is driven exclusively by memory autoreactive T-cells that had been primed during the development of spontaneous autoimmune diabetes. Our findings underscore the need for clinical treatment strategies managing chronic auto-immunity.

Supported by: Canadian Diabetes Association, Dutch Diabetes Research Foundation

## PS 022 Intervention in type 1 diabetes

452

### Metabolic parameters at baseline identify clinical responders to teplizumab 2 years after diagnosis of type 1 diabetes

K.C. Herold<sup>1</sup>, M. Ehlers<sup>2</sup>, K. Boyle<sup>3</sup>, J. McNamara<sup>4</sup>, S. Gitelman<sup>5</sup>, P. Gottlieb<sup>6</sup>, C. Greenbaum<sup>7</sup>, W. Hagopian<sup>8</sup>, J.A. Bluestone<sup>9</sup>;

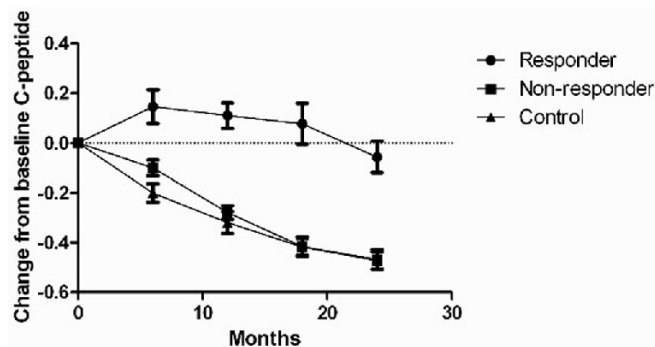
<sup>1</sup>Immunobiology, Yale University, New Haven, <sup>2</sup>Immune Tolerance Network, San Francisco, <sup>3</sup>Rho Inc, Chapel Hill, <sup>4</sup>NIAID, Bethesda, <sup>5</sup>Pediatrics, UCSF, San Francisco, <sup>6</sup>Medicine, University of Colorado, Aurora, <sup>7</sup>Benaroya Research Institute, Seattle, <sup>8</sup>Pacific Northwest Research Institute, Seattle, <sup>9</sup>Medicine, UCSF, San Francisco, USA.

**Background and aims:** Some patients with T1D, treated with humanized FcR non-binding anti-CD3 mAb, teplizumab, have had improved C-peptide responses to a mixed meal for as long as 2 years after diagnosis but not all trials have achieved their endpoint and parameters that can identify individuals before treatment most likely to respond to therapy have not been identified.

**Materials and methods:** We conducted an open-labelled trial of teplizumab in 83 patients with new onset T1D. Patients were randomised to treatment with two 14-day courses of teplizumab, 1 year apart, or intensive diabetes care, in a 2:1 ratio. The endpoint was a comparison of the change from baseline of the mean stimulated C-peptide 4-hour AUC responses to a mixed meal after 2 years. We identified clinical responders (post-hoc) as individuals whose C-peptide AUC at 24 months had fallen by  $\leq 40\%$  of the response at study entry.

**Results:** The intention to treat analysis included 52 drug-treated and 25 control subjects; of the drug-treated subjects, 40 received all/part of the 2<sup>nd</sup> drug cycle. Teplizumab treatment significantly reduced the decline in baseline C-peptide at month 24 (primary endpoint:  $-0.283$  ( $0.3155$ ) vs.  $-0.458$  ( $0.1555$ ) pmol/mL,  $p=0.002$ ) and reduced insulin use over 2 years ( $p=0.036$ ) to achieve HbA<sub>1c</sub> levels that were not significantly different ( $p=0.697$ ). A greater percentage of control subjects (33%) lost detectable stimulated C-peptide compared to teplizumab treated subjects (4%,  $p=0.001$ ). Although the C-peptide AUC responses represented a continuum, 22/52 of the drug-treated but none of the control subjects lost  $\leq 40\%$  of responses at 24 months. In the responding subjects, the C-peptide responses at 12, 18, and 24 months were 114%, 108%, and 88% of baseline whereas in the non-responders the responses were 57%, 39%, and 29% of baseline responses ( $p<0.001$  at all). The clinical responders were indistinguishable from the non-responders in age, weight, BMI, sex, duration of disease, autoantibodies, and baseline C-peptide responses. However, they had a lower baseline HbA<sub>1c</sub> ( $7.05$  ( $0.815$ ) vs  $7.71$  ( $1.030$ ) %,  $p<0.016$ ) and insulin dose ( $0.24$  ( $0.138$ ) vs  $0.46$  ( $0.246$ ) U/kg,  $p<0.005$ ).

**Conclusion:** Teplizumab treatment of patients with new-onset T1D significantly improves C-peptide response for at least 2 years after disease onset. A group of clinical responders who show little decline in C-peptide over this time have lower HbA<sub>1c</sub> and insulin use at diagnosis. These findings suggest metabolic memory that may affect responses to immune therapies.



Clinical Trial Registration Number: NCT00129259

Supported by: NIAID/NIH/JDRF

453

### Effects of the GLP-1 receptor agonist liraglutide on human peripheral immune cells from healthy subjects in vitro and in vivo

G. Bock<sup>1</sup>, B. Prietl<sup>1</sup>, M. Tauschmann<sup>1</sup>, E. Höller<sup>1</sup>, C. Neuper<sup>1</sup>, W. Graninger<sup>2</sup>, T.R. Pieber<sup>1,3</sup>;

<sup>1</sup>Endocrinology and Metabolism, University Hospital Graz, <sup>2</sup>Rheumatology and Immunology, University Hospital Graz, <sup>3</sup>HEALTH - Institute of Biomedicine and Health Science, Joanneum Research, Graz, Austria.

**Background and aims:** Glucagon-like peptide-1 receptor agonists (GLP-1RA) are used as a novel therapy in type 2 diabetes. Treatment with GLP-1RA in T2D patients with psoriasis suggested immunomodulatory effects of GLP-1RA and anti-inflammatory effects in preclinical studies had been observed. In this study, we investigated the in vitro effect of liraglutide on human immune cells as well as the in vivo effect on the frequency of immune competent cells in the peripheral blood of healthy subjects.

**Materials and methods:** Proliferation of PBMCs from healthy donors was tested in vitro after exposing cells to increasing concentrations of liraglutide (0–80 µg/ml). Unstimulated cells and cells stimulated by adding anti-CD3/CD28 dynabeads were exposed to liraglutide and tested for their proliferation after 96 h. In vivo tests were done in a pilot trial including 5 healthy subjects (2f/3m; age:  $35\pm9$  years; BMI:  $25.1\pm4.9$ ) who were initially treated with 0.6 mg liraglutide/day followed by 1.2 mg/day for a total of 4 weeks. A multi-color FACS analysis for Treg, B-, iNKT-, NKT-, NK-, Th1-, Th2-, Th17 cells and DCs was performed at baseline (BL), after 2 and 4 weeks.

**Results:** In vitro PBMC proliferation of unstimulated cells increased 2–3 fold upon addition of liraglutide (20, 40, 80 µg/ml) whereas proliferation of stimulated cells did not change. In vivo peripheral Treg in CD4<sup>pos</sup>Tc increased significantly from  $4.64\pm1.06$  (BL) to  $5.74\pm1.14$  after 2 wks ( $p=0.001$ ) and  $5.52\pm0.83$  after 4 wks ( $p=0.004$ ) whereas Helios expression remained stable. The frequency of Th1-cells decreased significantly ( $7.50\pm2.42$  at baseline to  $4\pm1.96$  after 4 weeks,  $p=0.002$ ) whereas all other cell types remained stable.

**Conclusion:** Our study is first to show immunomodulatory effects of liraglutide treatment in healthy human subjects and our results advance the mechanistic insight into the immunomodulatory potential of incretin hormones in vitro and in vivo. Our findings provide further rationale to investigate potential beneficial effects of GLP-1RA treatment in autoimmune diseases such as T1D.

Supported by: EFSD/MSD grant

454

### Effect of anti-thymocyte globulin (ATG) on preserving beta cell function in new-onset type 1 diabetes

S.E. Gitelman<sup>1</sup>, L.K. Fisher<sup>2</sup>, P.A. Gottlieb<sup>3</sup>, M. Gottschalk<sup>4</sup>, W.V. Moore<sup>5</sup>, A. Moran<sup>6</sup>, M.R. Rigby<sup>7</sup>, S.M. Willi<sup>8</sup>, L. Keyes-Elstein<sup>9</sup>, A. Pinckney<sup>9</sup>, L. Ding<sup>10</sup>, M.R. Ehlers<sup>11</sup>, START Study Team;

<sup>1</sup>Pediatric Diabetes Program, University of California San Francisco, <sup>2</sup>Endocrinology, Children's Hospital of Los Angeles, <sup>3</sup>Pediatrics, Barbara Davis Center, Aurora, <sup>4</sup>Pediatric Endocrinology, University of California San Diego, <sup>5</sup>Endocrine/Diabetes, Children's Mercy Hospital, Kansas City, <sup>6</sup>Pediatric Endocrinology, University of Minnesota, Minneapolis, <sup>7</sup>Pediatrics, Indiana University, Indianapolis, <sup>8</sup>Pediatrics, Children's Hospital of Philadelphia, <sup>9</sup>Biostatistics, RhoFed, Inc., Chapel Hill, <sup>10</sup>DAIT, National Institute of Allergy and Infectious Diseases, Bethesda, <sup>11</sup>Clinical Trials Group, Immune Tolerance Network, San Francisco, USA.

**Background and aims:** Type 1 diabetes (T1D) results from T-cell mediated destruction of insulin-producing beta cells. Prior preclinical and clinical studies show that therapies targeting T-cells preserve beta cell function. Most promising are results with anti-thymocyte globulin (ATG): several doses can induce a lasting remission in the NOD mouse, and several smaller pilot clinical studies with ATG alone or in combination with other drugs preserve endogenous insulin secretion. We now report the results of a two-arm, randomized, placebo-controlled, blinded, phase 2 clinical trial in recent-onset T1D.

**Materials and methods:** Subjects aged 12–35 were screened and enrolled within 100 days of T1D diagnosis. They were randomized 2:1 to ATG or placebo, and underwent identical procedures throughout the trial. Drug/placebo was administered over a 5-day observation period in a clinical research center; those in the treatment group received a total ATG dose of 6.5 mg/kg. Subjects were followed closely over the ensuing 2 years. Primary endpoint is endogenous insulin secretion, as assessed by 2-hour C-peptide AUC in

response to a mixed-meal tolerance test at month 12. Secondary measures include safety experience, additional metabolic measures, and immunologic studies to assess mechanism of action. The sample size is calculated to have 80% power to detect 50% improvement in the treatment arm (2-sided t-test, alpha 5%, assuming 10% drop-out rate).

**Results:** 58 subjects were randomized to the trial, with median age of 17.5 years, 35 males, and 49 with primary race of white. All except 2 subjects completed the full study drug infusion course. 29 serious adverse events were reported for 13 subjects; 7 events were attributed to study drug. Cytokine release syndrome or serum sickness was noted in 38 subjects, and was managed with glucocorticoids for < 2 weeks duration. 38 subjects also exhibited decreased lymphocyte or CD4 counts; 68% had CD4 counts recover to > 200 x 10<sup>6</sup>/L by month 6, and 91% by month 12. No unusual or unexpected infections were noted, nor was cytomegalovirus or Epstein Barr virus reactivation observed. The primary endpoint data and additional secondary measures will be available in June, 2012, and will be reported at the time of the meeting.

**Conclusion:** ATG is a potential means to arrest beta cell destruction and induce tolerance for subjects with T1D. Mechanistic studies are underway to determine how this drug functions in vivo, with prior studies suggesting depletion of autoreactive T cells coupled with induction of regulatory T cells. Long term follow-up is ongoing to assess the durability of response.

*Clinical Trial Registration Number:* NCT00515099

*Supported by:* ITN, NIAID, NIDDK, JDRF

## 455

### Evaluation of treatment effect of a type 1 diabetes intervention therapy using GST versus MMTT

S. Dagan<sup>1</sup>, D. Peled<sup>1</sup>, D. Elias<sup>1</sup>, A. Avron<sup>1</sup>, R. Eren<sup>1</sup>, I. Raz<sup>2</sup>, P. Pozzilli<sup>3</sup>; <sup>1</sup>Andromeda Biotech Ltd., Yavne, Israel, <sup>2</sup>Diabetes Unit, Department of Medicine, Hadassah University Hospital, Jerusalem, Israel, <sup>3</sup>Universita Campus Bio-Medico, Rome, Italy.

**Background and aims:** Endogenous insulin secretion by pancreatic beta cells is measured by stimulated C-peptide area under the curve (AUC). C-peptide stimulation is achieved by either intravenous administration of glucagon (GST) or ingestion of a mixed meal (MMTT), two standard assays that are commonly used. These procedures were compared previously in terms of sensitivity, reproducibility, and tolerability. However, a comparison between the two methods in measuring the treatment effect of type 1 diabetes (T1D) interventions, evaluated by the change in C-peptide AUC during the treatment period, was not performed. Our goal is to compare the changes in C-peptide AUC from baseline to end of treatment as measured by GST and MMTT.

**Materials and methods:** A phase III clinical study, DIA-AID 1, was conducted to evaluate safety and efficacy of DiaPep277 in preserving beta cell function in newly diagnosed T1D patients. We compared the C-peptide values of 151 patients in the placebo arm who had the AUC measured at least at baseline and after 24 months and evaluated the change from baseline to 24 months of C-peptide AUC using GST and MMTT.

**Results:** We correlated C-peptide AUC and the change from baseline AUC ( $\Delta$ AUC), measured by GST and MMTT in each individual patient at different time points. When comparing the AUC, the correlation coefficients range between 0.73–0.86 with time, improving at 24 months as compared to baseline. However, when comparing the  $\Delta$ AUC, we found no correlation between the two assays in all time points (correlation coefficients range between 0.35–0.57). Correlation between the change in fasting C-peptide ( $\Delta$  Fasting C-peptide) and change in AUC ( $\Delta$  AUC) from baseline to 24 months, measured by each of the two assays was better when using the GST. The clinical outcomes from the DIA-AID 1 study, HbA1c, insulin dose and number of hypoglycemic events at study end, support the finding that DiaPep277 led to a significant treatment effect in C-peptide  $\Delta$ AUC ( $p=0.035$ ) as measured by GST.

**Conclusion:** Although both GST and MMTT are reproducible and correlate well in evaluating absolute levels of C-peptide, there is a significant difference between the two tests in terms of measuring the change in C-peptide levels overtime. Further studies should be conducted to explore the nature of these discrepancies.

*Clinical Trial Registration Number:* NCT006 15 264

## 456

### Functional modulation of epidermal dendritic cells by topical steroid treatment to optimise the tolerogenic potential of immunotherapy for type 1 diabetes

M. Alhadj Ali, S. Hanna, S. Wong, C. Dayan;

Centre for Endocrine and Diabetes Sciences, Cardiff University, UK.

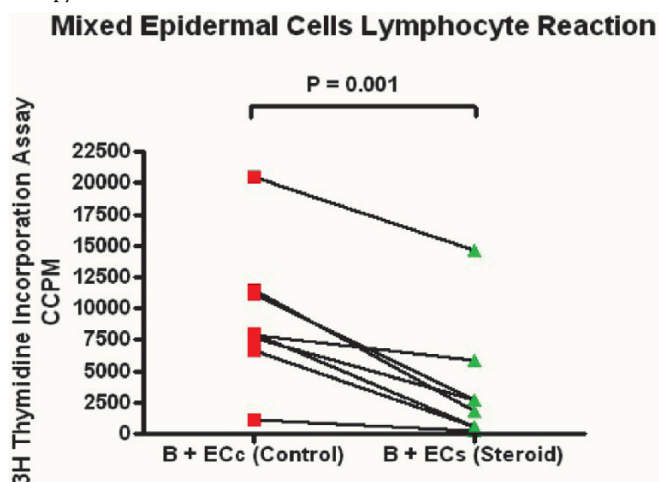
**Background and Introduction:** Administration of short peptides corresponding to T cell target sequences (peptide immunotherapy) has been shown to be a simple and effective method of restoring tolerance and reversing disease in animal models of T1D and has several potential advantages over other approaches to Antigen Specific Immunotherapy. In humans use of the intradermal route has a specific advantage in peptide immunotherapy as dendritic cells are potentially accessible for both peptide immunotherapy and local treatment with agents able to maintain the tolerance state.

**Aims and objectives:** We aimed to determine whether topical pretreatment of the skin with Betamethasone has the potential to enhance the tolerogenic response in peptide immunotherapy.

**Materials and methods:** 8 healthy volunteers received 0.05% Betamethasone twice daily for 4 days to the skin of a small area of the left arm. At day 5, all volunteers had a 10–15mm suction blister raised at the site of treatment and a second (control) blister raised in the right arm. Epidermal cells from treated and untreated skin were used separately to stimulate Mixed Epidermal Cells Lymphocyte Reaction (MECLR) using allogeneic PBMCs from healthy donor (B) and used also in a 14 days culture in combination with CD4+ CD45RA+ naive T cells from (B). T cells recovered from these cultures were added to a fresh MLR response to test their suppressive capacity. Natural T Regs (CD4+ CD25+ CD127 low) were separated from donor (B) and used as a positive control to suppress the MLR. PBMCs taken from the blister donor were used also in combination with PBMCs from (B) to stimulate a Mixed Lymphocyte Reaction (MLR).

**Results:** MECLR responses driven by epidermal cells from the steroid treated arm were 60.9% lower than with cells from the untreated arm ( $P=0.001$ ). MECLR responses were “normalized” to the MLR response and MECLR/MLR was also markedly reduced in the steroid treated arm compared with the ratio calculated from the control arm (60.3% difference -  $P=0.008$ ). After 14 days culture, T cells recovered from culture with epidermal dendritic cells (EDC) from the steroid treated arm markedly tended to suppress a fresh MLR response more than T cells induced by culture containing (EDC) from the control arm (Mean reduction=61.86% with SD=36.35%). MLR responses with NTregs separated from (B) were 89% lower than with T cells recovered from culture with untreated epidermal dendritic cells (SD= 5.88%).

**Conclusion:** Pretreatment of the skin with Betamethasone for as little as 4 days has significant effects on epidermal dendritic cells function shown in the Mixed Epidermal Cells Lymphocyte Reaction. The pilot functional studies indicate the ability of steroid treated epidermal dendritic cells to induce T cells which are able to suppress the proliferation of autologous naive T cells. Further functional assays are required to determine whether these represent T Reg cells and the mechanism of suppression induced by steroid topical therapy.



*Clinical Trial Registration Number:* CT646

*Supported by:* Novo Nordisk Research Foundation, Action Medical Research and EU NAIMIT



## 457

**Metabolic and immunologic observations from a trial of alpha -1 antitrypsin in recent onset type 1 diabetes**

P. Gottlieb, A. Michels, D. Di Domenico, L. Fitzgerald-Miller, K. Johnson, J. Lungaro, L. Meyers, A. Conley, D. Zipris;  
Pediatrics/Medicine, Barbara Davis Center, Aurora, USA.

**Background and aims:** Immune therapy of type 1 diabetes (T1D) to date has focused on therapies to affect T lymphocytes. These therapies have been able to interrupt the disease process, but in time it resumes and destruction of beta cells and loss of C-peptide ensues. Inflammation may be playing a role in setting the milieu which propagates and allows the autoimmune process to be maintained. Alpha-1 antitrypsin (AAT) has been shown to be effective in the NOD mouse and islet transplantation models where it has an ability to downregulate many pro-inflammatory pathways. This study was designed as a pilot to understand the metabolic and immunologic effect of AAT in recent onset T1D.

**Materials and methods:** This study was a single center, open label, safety and efficacy study where individuals who were autoantibody positive, within 5 years of diagnosis who retained measurable C-peptide were given 8 weekly infusions of AAT at 80/mg/kg. Endpoints included safety, AAT levels, metabolic endpoints as well as inflammatory and T cell markers. A mixed meal test was performed at baseline, 3, 6, 9 and 12 months and the 2 hour C-peptide AUC was assessed.

**Results:** 12 subjects were enrolled in the trial and all subjects completed their infusions. There were mild AE's observed which included headache and hypoglycemia. No SAE's were observed. We divided subjects into those with >1 year duration vs. those with < 1 year duration. We saw some drop in C-peptide among the 4 subjects with >1 year duration, while insulin use and HbA1c was essentially unchanged during the 3 months of therapy. In contrast for those with <1 year duration, we saw 4 out of 7 subjects had an increase in C-peptide at 3 months, while 3 of 7 decreased their insulin use as well. HbA1c remained well controlled throughout the treatment period. Trough AAT levels were progressively raised throughout the 8 weeks of treatment, but did not exceed the normal range at the doses used in this study. Immunologic assays to look at modulation of TLR-induced inflammatory signals in monocyte and dendritic cells failed to demonstrate a consistent effect of AAT.

**Conclusion:** AAT was well tolerated and safe in the doses used in the study. There was suggestion of metabolic effect of the study drug in those with more recent onset, but it is not clear how that effect is being mediated from this pilot trial. Further trials with more subjects, additional doses and additional biomarkers are warranted to better understand the role and effect of anti-inflammatory therapy in T1D.

Clinical Trial Registration Number: NCT01319331

Supported by: Omni Bio Pharmaceutical Inc

## 458

**Therapeutic effects produced by administration of human preproinsulin gene in rodents with experimental type 1 diabetes mellitus**

M.D. Tronko<sup>1</sup>, O.I. Kovzun<sup>1</sup>, L.N. Kalynska<sup>1</sup>, I.P. Paster<sup>1</sup>, V.A. Kordum<sup>2</sup>, T.P. Gulko<sup>2</sup>, O.K. Toropova<sup>2</sup>;

<sup>1</sup>State Institution "V.P.Komisarenko Institute of Endocrinology and Metabolism of Natl. Acad. Med. Sci. Ukraine", <sup>2</sup>Institute of Molecular Biology and Genetics of Natl. Acad. Sci. Ukraine; State Institution, Kyiv, Ukraine.

**Background and aims:** Insulin-dependent diabetes mellitus is associated with an almost complete destruction of insulin-producing pancreatic beta-cells, which leads to insulin deficiency in the body. A radical treatment of diabetes may be provided by gene therapy - namely, by administration to patients of a human insulin gene in molecular construction that would provide its expression in non-specific cells which do not produce endogenous insulin.

**Materials and methods:** The molecular vector construction consisted of two independent modules: a bacterial plasmid backbone allowing its replication in *Escherichia coli* cells, and an expression cassette with a target full-size gene of preproinsulin, flanked by inverted terminal repeats of human adeno-associated virus 2. Human cytomegalovirus immediate-early promoter was used to drive ectopic insulin transgene expression in liver cells. A branched polyethyleneimine (25 kD) has been used to transfect liver cells *in vivo*. We have used a model of diabetes in Wistar rats and C57BL/6j mice to study the effects of recombinant molecules that contain a target gene of human preproinsulin. Streptozotocin (STZ) (Sigma, U.S.A.) was administrated to male animals aged 2 to 2.5 months every day for 5 days at a dose 50 mg/kg. The signs of STZ diabetes development in animals were a loss of weight, an increased blood

glucose level and polyuria. One month after STZ administration, animals with developed hyperglycemia (17.5 to 29.2 mmol/L) were divided into three groups: diabetic animals which were injected with vector DNA containing human preproinsulin gene, and two control groups: diabetic animals which did not receive DNA and diabetic animals which received control plasmid DNA without target gene. DNA/polyethyleneimine complexes for injections were prepared in a weight equivalent 1:3. DNA amount in transfection preparation was 10 µg per 20 g of weight. The therapeutic effect of injected recombinant molecules was assessed according to glucose content in blood plasma of experimental animals - by glycemia level four hours after the last meal. Glucose was tested using a test-system "Hemoglan" from ("Norma", Ukraine) and on an analyzer "SUPER GL" (Germany).

**Results:** The data obtained revealed a significant decrease in blood glucose level in 85% of experimental animals after preproinsulin gene administration. The tendency to glucose levels decrease has also been revealed in experiments with plasmid control. Within 5 to 12 weeks after STZ administration the majority of animals from control groups have demonstrated high glycemia levels, loss of weight, polyuria, and a high lethality rate which exceeded by several times the rate for experimental animals. The decrease in blood glucose level (22.4 to 13.3 mmol/L) with term 2.5-5 weeks was shown in diabetic rats (5 weeks diabetes) 1 week after molecular vector DNA administration.

**Conclusion:** These results show that regression of diabetes has been achieved after a single course of gene therapy on the model of experimental type 1 diabetes in rats and mice. This approach could be used to study islet physiology and to assay new gene therapy approaches for diabetes mellitus.

Supported by: Government of Ukraine

## 459

**11-Keto-β-boswellic acid prevents development of insulinitis in NOD-mice**

H.P.T. Ammon<sup>1</sup>, A.M. Shehata<sup>2</sup>, J. Jauch<sup>3</sup>, L. Quintanilla-Martinez<sup>4</sup>;  
<sup>1</sup>Pharmacology and Toxicology, Pharmaceutical Institute, University of Tübingen, Germany, <sup>2</sup>Pharmacology and Toxicology, Faculty of Pharmacy, University of Beni-Suef, Egypt, <sup>3</sup>Organic Chemistry, Institute of Organic Chemistry, University of Saarland, Saarbrücken, Germany, <sup>4</sup>Pathology, Institute of Pathology, University of Tübingen, Germany.

**Background and aims:** 11-Keto-β-boswellic acid (KBA) has been shown to prevent insulinitis in the model of Multiple Low Dose-Streptozotocin (MLD-STZ) diabetic mice. Here in a second model of autoimmune diabetes which is the Non Obese Diabetic (NOD) mouse it should be tested whether or not KBA produces similar effects compared to the MLD-STZ diabetes.

**Materials and methods:** Female NOD mice 4 weeks of age (Charles River) were i.p. injected for 3 weeks with 7.5 mg/kg KBA. After 3 weeks of KBA-treatment, animals were killed and blood was collected for determination of glucose and cytokines (IL-1A, IL-1B, IL-2, IL-6, IFN-γ, TNF-α). Pancreases were isolated for histochemistry. Another group of mice was followed up for monitoring of blood glucose and body weight for 22 weeks. Cytokines were analyzed using Multi-Analyte ELISArray<sup>™</sup>. Lymphocytes infiltration into pancreatic islet cells and apoptosis were detected with anti-CD3 and anti-caspase 3 respectively. Blood glucose was evaluated with test strips (Accu-check aviva glucometer).

**Results:** In the seventh week of age there was infiltration of lymphocytes and appearance of apoptotic cells in pancreatic islets of all control mice. Cytokines in the blood showed no change at this time, but blood glucose was significantly decreased (106.5±4.6 vs. 77.3±5.4 mg/dl, P<0.05). From 6 animals treated with KBA 4 showed no infiltration of lymphocytes into pancreatic islets as well as no appearance of apoptotic islet cells in all while blood glucose levels did not change. Furthermore, there was no change of cytokines in comparison to control. After 12 weeks control group still exhibited decrease of blood glucose (71.7±2.9 mg/dl, P<0.05) and body weight was not increased compared to four weeks old animals (16.5±0.4 vs. 15.0±0.8). However, in KBA treated animals body weight increased (17.8±0.4 vs. 23.2±0.6, P<0.05) and blood glucose level was still normal (91.5±3.0 vs. 100.2±4.5 mg/dl). After the week 18, in 4 of 5 animals there was a sharp increase of blood glucose in the non treated animals, while on the other hand only 3 out of 6 KBA treated mice developed hyperglycemia.

**Conclusion:** Similar to recent results obtained in mice with MLD-STZ-diabetes, KBA reduced development of insulinitis and apoptosis of islet cells of NOD mice. In this period the hypoglycemia appearing in control NOD mice which may be due to insulin leakage from damaged β-cells. This could be prevented by KBA treatment. Moreover, KBA treatment of NOD mice allowed normal development of body weight over the period tested. Since so far KBA was effective in two different animal models of type 1 diabetes, the data are promising for clinical studies in autoimmune diabetes.

## PS 023 Functional beta cell mass in type 1 and type 2 diabetes

460

### BMI explains the major part of differences in beta cell function and insulin sensitivity between Japanese and Caucasians

M. Ohsugi<sup>1</sup>, H. Tanaka<sup>1</sup>, M. Pedersen<sup>2</sup>, J.B. Møller<sup>3</sup>, R.V. Overgaard<sup>3</sup>, J. Lyng<sup>3</sup>, K. Almind<sup>3</sup>, N.-M. Vasconcelos<sup>3</sup>, P. Poulsen<sup>3</sup>, C. Keller<sup>3</sup>, K. Ueki<sup>1</sup>, S.H. Ingwersen<sup>3</sup>, B.K. Pedersen<sup>2</sup>, T. Kadowaki<sup>1</sup>

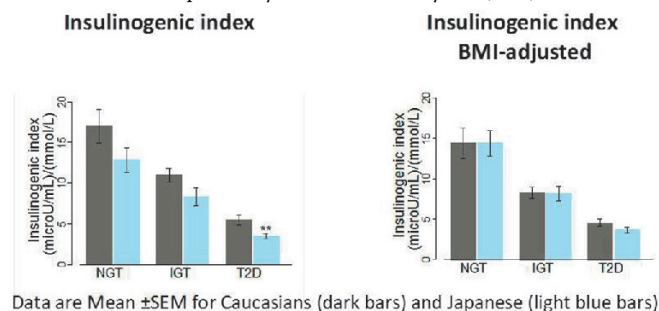
<sup>1</sup>Department of Metabolic Diseases, University of Tokyo, Japan, <sup>2</sup>Centre of Inflammation and Metabolism, Department of Infectious Disease, Rigshospitalet, University of Copenhagen, Denmark, <sup>3</sup>Novo Nordisk A/S, Bagsvaerd, Denmark.

**Background and aims:** Ethnic differences in the pathophysiology of type 2 diabetes (T2D) have been the subject of several investigations. Japanese individuals are perceived to have higher insulin sensitivity and less ability to compensate for insulin resistance by increased beta-cell function compared to Caucasians. However, the data to support this are limited. We undertook a cross-sectional study in Japanese and Caucasians with the aim of investigating beta-cell function and insulin sensitivity and the contribution of demographic, genetic, biochemical and life-style related factors to differences found.

**Materials and methods:** The study comprised 120 Japanese and 150 Caucasians ranging from normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) to T2D with similar distributions between high/low BMI in each of the glucose tolerance groups. BMI cut-off values were set in accordance with regional demographic differences. Beta cell function (HOMA-B, insulinogenic index and insulin secretion ratio) and insulin sensitivity (HOMA-IR and Matsuda index) were assessed from oral glucose tolerance tests and analysed by ANOVA.

**Results:** Mean glucose profiles were similar in Japanese and Caucasian NGT, IGT, and T2D subjects, respectively whereas insulin and C-peptide responses were lower in Japanese. Japanese were less insulin resistant than Caucasians and the beta-cell function was lower in Japanese compared to Caucasians. The disposition index - a measure of beta cell function relative to insulin resistance - was similar between Japanese and Caucasians in each glucose tolerance group. Among the covariates studied, BMI and other measures of body size, such as waist and weight, were major determinants of both insulin sensitivity and beta cell function. In this study, BMI was a design parameter and in order to quantify the role of BMI in explaining ethnic differences, endpoints were adjusted to the median BMI value for each glucose tolerance group. This was justified by similar linear relationships between individual endpoints and BMI values for the two ethnicities. Following BMI adjustment, the differences between Japanese and Caucasians were substantially reduced in all groups (shown for insulinogenic index in the figure). For all five indices, ethnic differences were no longer statistically significant for NGT and IGT participants.

**Conclusion:** We confirm that Japanese generally have higher insulin sensitivity and lower beta-cell function than do Caucasians. The major part of these differences can be explained by differences in body size (BMI).



Clinical Trial Registration Number: NCT00897169

Supported by: JPN Sci & Technol Agency, DK Agency Sci, Technol & Innov, Novo Nordisk

461

### Beta cell area and function in human type 2 diabetes and the relationships with anti-diabetic therapy

L. Marselli, M. Suleiman, M. Bugliani, F. Syed, F. Scatena, D. Focosi, L.M. Mariotti, F. Filipponi, U. Boggi, P. Marchetti;

Department of Endocrinology and Metabolism, University of Pisa, Italy.

**Background and aims:** Type 2 diabetes (T2D) is characterized by reduced beta cell functional mass. To date little information is available on the direct properties of human islets in this disease. Aim of the present study was to evaluate the amount of beta cells and their ex vivo function in non-diabetic (ND) and T2D subjects, to be correlated with clinical parameters, including anti-diabetic therapy in T2D patients.

**Materials and methods:** Pancreases were obtained from 288 ND donors (age: 59±17 yrs; 154 males and 134 females; BMI: 25.2±3.7 kg/m<sup>2</sup>), and 45 T2D subjects (age: 69±9 yrs; 23 males and 22 females; BMI: 27.7±4.1 kg/m<sup>2</sup>; mean duration of diabetes: 8±6 yrs). Beta cell amount was evaluated by insulin immunostaining and morphometric analysis; isolated islets were prepared by enzymatic digestion and density gradient purification; insulin secretion was assessed basally and after stimulation with glucose and arginine.

**Results:** Insulin positive area (IPA) per islet was lower in T2D cases (56±18% vs 71±12%, p<0.01). Insulin release in response to glucose (stimulation index, SI: 1.6±0.7 vs 2.9±1.8, p<0.05) and arginine (1.7±0.5 vs 2.4±1.4, p<0.01) was also significantly reduced in islets of T2D subjects. In T2D no correlation was observed between IPA or SI values and age, BMI or duration of diabetes; in addition, IPA and SI were not correlated each other. Forty-three % T2D subjects were treated with diet (D) or D plus metformin (group D+M), 40% received sulfonylurea alone or in combination with M (group S), the remaining 17% were treated with insulin either associated with oral hypoglycemic agents or alone (group I). The three therapy groups did not differ in terms of age and BMI. IPA was not significantly different between D+M (47±18%), S (66±14%) and I (55±18%) groups. Glucose SI was higher in D+M (2.0±0.8, p<0.05, Bonferroni corrected) than S (1.4±0.4, p<0.05) and I (1.0±0.1, p<0.05) islets. No difference was observed between groups as for arginine SI.

**Conclusion:** These results confirm that islets of T2D subjects have reduced beta cell amount and function, which in our cohort were not significantly correlated with age, BMI, and duration of diabetes; the type of anti-diabetic therapy was not associated with the amount of islet beta cells, but rather with the residual beta cell glucose competence. Rescuing beta cell function thus appears to be a primary target of T2D therapy.

462

### Change in beta cell and alpha cell mass in Japanese obese individuals

K. Kou<sup>1</sup>, Y. Saisho<sup>1</sup>, T. Yamada<sup>2</sup>, H. Itoh<sup>1</sup>;

<sup>1</sup>Internal Medicine, Keio University School of Medicine, <sup>2</sup>Pathology, Keio University School of Medicine, Tokyo, Japan.

**Objective:** Both type 1 and type 2 diabetes are characterized by a deficit of β-cell mass. Thus to increase β-cell mass is an important strategy for the treatment of both types of diabetes. Although it has been reported that β-cell mass increases approximately 50% in response to obesity in Caucasian, there is limited data available as to β-cell mass in Asian population. Less degree of obesity with similar incidence of type 2 diabetes in Asian compared to Caucasian suggests less functional β-cell capacity in Asian. Therefore in this study we examined whether β-cell mass increases in Japanese obese individuals.

**Research design and methods:** We obtained autopsy pancreas from 30 lean (defined as BMI 23.5 or less) and 17 obese (defined as BMI 25 or more) Japanese adults aged from 20 to 60 years (age 47 ± 12 (mean ± SD) vs. 44 ± 12 years, p = 0.44, BMI 20.2 ± 1.3 vs. 27.2 ± 1.6, p < 0.0001, respectively). To be included, cases were required to have 1) had a full autopsy within 24 h of death, 2) pancreas tissue stored with adequate size and quality. Cases were excluded if 1) subjects had been diagnosed as diabetes or exposed to chronic glucocorticoid treatment, 2) pancreas tissue showed autolysis or any abnormal change such as pancreatitis. Pancreas tissues were stained for insulin or glucagon, and fractional β-cell area and α-cell area to the pancreas (%) were measured, respectively. β-cell and α-cell mass was also calculated as a product of fractional β-cell area and reference values of pancreas parenchymal mass. We also measured frequency of β-cell replication and apoptosis by immunohistochemistry for Ki-67 staining and ssDNA staining, respectively. β-cell neogenesis was evaluated by density of insulin positive duct cell. In addition, we measured β-cell size in each case.

**Results:** There was no increase in fractional  $\beta$ -cell area ( $1.6 \pm 0.9$  vs.  $1.1 \pm 0.8$  %,  $p = 0.05$ ) and fractional  $\alpha$ -cell area ( $1.1 \pm 0.8$  vs.  $0.9 \pm 0.7$  %,  $p = 0.29$ ) in the obese individuals compared with the lean individuals. These results did not change even when  $\beta$ -cell mass ( $0.74 \pm 0.40$  vs.  $0.54 \pm 0.39$  g,  $p = 0.11$ ) and  $\alpha$ -cell mass ( $0.48 \pm 0.26$  vs.  $0.43 \pm 0.31$  g,  $p = 0.52$ ) were used in the analysis. As a result,  $\alpha$ -cell area to  $\beta$ -cell area ratio was not changed in obesity ( $0.77 \pm 0.61$  vs.  $1.15 \pm 1.26$ ,  $p = 0.17$ ), while  $\alpha$ -cell area is significantly positively correlated with  $\beta$ -cell area ( $r = 0.44$ ,  $p = 0.002$ ). There was no significant difference in mean  $\beta$ -cell size between the groups ( $8.6 \pm 0.8$  vs.  $8.8 \pm 0.6$   $\mu\text{m}$ ,  $p = 0.33$ ). The frequency of  $\beta$ -cell apoptosis was very low in all cases and there was no difference in the frequency of  $\beta$ -cell apoptosis between the groups. There was no difference in the frequency of  $\beta$ -cell replication between the groups ( $0.039 \pm 0.045$  vs.  $0.043 \pm 0.041$  /islet,  $p = 0.77$ ), while the frequency of  $\beta$ -cell replication was positively correlated with fractional  $\beta$ -cell area ( $r = 0.48$ ,  $p = 0.001$ ). There was no difference in insulin positive ductal cell density between the groups ( $0.05 \pm 0.1$  vs.  $0.09 \pm 0.03$  /mm<sup>2</sup>,  $p = 0.14$ ).

**Conclusion:**  $\beta$ -cell mass was not increased in Japanese obese individuals. There was a positive correlation between fractional  $\beta$ -cell area and  $\beta$ -cell replication, suggesting that regulation of  $\beta$ -cell mass might be determined by  $\beta$ -cell replication in adult Japanese, which, however, may not be upregulated in obesity. Our findings suggest the possibility that  $\beta$ -cell regenerative capacity in Japanese may differ from Caucasian population. Comparison of islet morphology among different ethnics may provide insight into the mechanism of  $\beta$ -cell regeneration.

*Supported by: NMT Research and Education Fund, DS Health Foundation*

## 463

### The prevalence of endogenous insulin production in a cohort of adults with type 1 diabetes

R.A. Oram, M.H. Shepherd, M. Hudson, T. Sanders, S. Tiley, S. Hammersley, B. Shields, T.J. McDonald, B.A. Knight, R.E.J. Besser, A.T. Hattersley;  
Institute of Biomedical & Clinical Science, Exeter, UK.

**Background and aims:** The practical difficulty of serum C-peptide measurement means there are few community studies of endogenous insulin production in Type 1 Diabetes (T1D). We aimed to use urine C-peptide creatinine ratio (UCPCR) to quantify the prevalence and associated clinical features of insulin production in a cohort of adults with T1D. We also aimed to quantify how many with an initial diagnosis of T1D had an alternate diagnosis such as MODY or Type 2 Diabetes (T2D).

**Materials and methods:** 386 Subjects (180 male) were recruited to the UNITED project. All were on insulin from diagnosis, diagnosed <30 years, and current age <50 years. Insulin production was assessed using 2 hour post-prandial UCPCR. The presence of significant insulin production was assessed using a cut-off of 0.2 nmol/mmol. If UCPCR was >0.2 nmol/mmol then antibody testing for GAD and IA2 was performed. If GAD or IA2 antibodies were present then the diagnosis was assumed to be T1D, if they were not present then genetic testing was performed to rule out MODY. In patients with a negative genetic test for MODY a BMI cut off of 30 was applied before assuming the diagnosis was T1D.

**Results:** 345/386 (90%) had C-peptide detectable in the urine (UCPCR >0.01 nmol/mmol) 70/386 (18%) had a UCPCR >0.2 nmol/mmol. 35/70 patients with a UCPCR >0.2 nmol/mmol were antibody positive and assumed to have T1D. 9 of the remaining 35 were found to have MODY on genetic testing (8 HNF1A, 1 GCK and 1 HNF1B). 6 more patients were excluded from analysis with a BMI >30. Therefore a total of 15/386 (4%) of patients had an alternate diagnosis. The remaining group of 371 were assumed to have T1D. 55/371 (15%) had a UCPCR >0.2 nmol/mmol and 307/371 (83%) had UCPCR >0.01. 78% of patients (29/37) within 5 years of diagnosis had a UCPCR >0.2. 8% (27/334) of patient >5 years post diagnosis and 5% (8/186) >20 years post diagnosis had a UCPCR >0.2 nmol/mmol. Patients with UCPCR >0.2 had a lower 24 hour insulin dose ( $0.6$  v  $0.7$  u/Kg,  $p < 0.0001$ ) and shorter duration ( $5$  v  $21$  years,  $p < 0.0001$ ). Although there was no difference between HbA1c ( $8.8$  v  $8.6$   $p = 0.7$ ).

**Conclusion:** UCPCR allows screening of large populations of patients for residual insulin production. 18% of patients with an initial diagnosis of T1D were making significant amounts of insulin in this study. 4% of all patients were misdiagnosed and had MODY or T2D. In the refined T1D group 78% < 5 years and 8% >5 years post diagnosis had significant endogenous insulin. 5% of patients with T1D >20 years duration had significant endogenous insulin.

*Supported by: WELLCOME/DUK*

## 464

### Persistence of C-peptide secretion and impact on health in advanced type 1 diabetes

D.L. Faustman, L. Wang, S. Leung;  
Immunobiology Laboratory, Massachusetts General Hospital, Charlestown, USA.

**Background and aims:** In patients with longstanding disease, even modest beta-cell function is associated with a lower incidence of hypoglycemia, retinopathy and nephropathy compared to absent beta cell function when assessed using a standard C-peptide assay. This study examined the persistence of C-peptide secretion (a marker of insulin production and surviving beta cells) in the decades after type 1 diabetes onset and identified factors associated with preservation of beta-cell function in advanced disease.

**Materials and methods:** Using an ultrasensitive assay (lower detection limit: 1.5 pmol/L), we measured serum C-peptide levels in 182 human subjects with type 1 diabetes. Disease duration, age at onset, age, sex, and autoantibody titers were analyzed by regression analysis to determine their relationship to C-peptide production. A separate patient group (n=4) was serially studied for up to 20 weeks to examine C-peptide levels and beta cell functioning. Preliminary screening studies were also conducted to correlate remaining C-peptide with the prevention of hypoglycemic episodes.

**Results:** We report that the ultrasensitive assay detected C-peptide in 10% of individuals 31-40 years after disease onset; that patients with C-peptide levels as low as  $2.8 \pm 1.1$  pmol/L responded to hyperglycemia with increased C-peptide production, indicating intact beta cell functioning; that disease duration and level of zinc transporter 8 autoantibodies were significantly associated with C-peptide production; and that disease onset after age 40 was unexpectedly associated with low C-peptide production, despite shorter disease duration. New data will be presented on the status of low-level C-peptide secretion, assessed by ultrasensitive assay, as it relates to protection from hypoglycemia.

**Conclusion:** The ultrasensitive assay, 22x more sensitive than current standard assays, revealed that C-peptide production persists for decades after disease onset and remains functionally responsive in T1D. These findings suggest that patients with advanced disease, whose beta-cell function was thought to have long ceased, may benefit from interventions that preserve or stimulate beta cell function and should be more broadly included in clinical trials.

*Supported by: Iacocca Foundation*



## PS 024 Immune and viral correlates of beta cell failure

465

**Significance of lactoferrin autoantibodies in patients with type 1 diabetes**  
O.S. Derevyanko, T.V. Nikonova, E.V. Pekareva, A.V. Ilyin, O.M. Smirnova;  
Endocrinology Research Centre, Moscow, Russian Federation.

**Background and aims:** Histological analysis has demonstrated lymphocytic infiltration in endocrine and exocrine parts of pancreas in some patients with type 1 diabetes mellitus (T1DM). The aim of our study was to investigate the involvement of autoimmunity against the exocrine part of pancreas in T1DM patients and its association with other autoimmune diseases.

**Materials and methods:** 60 patients with T1DM and 20 healthy subjects (control group) were included in the study. None of them had any clinical symptoms of pancreatitis. The 1st group included 44 patients with only T1DM, the 2nd group - 16 patients with T1DM and autoimmune thyroiditis. Autoantibodies against lactoferrin (LACAb) were detected by using immunometric enzyme immunoassay. All subjects were tested for ICA, GADA, IA-2A, C-peptide, HbA1c.

**Results:** Significant difference was observed in frequency of LACAb occurrence between the study groups and control group ( $p < 0.05$ ). LACAb titer was significantly higher in patients of 1st and 2nd group than in control group ( $p < 0.05$ ). LACAb titer was 2,62 U/ml [2,02; 3,92] in 1st group; 3,31 U/ml [3,16; 3,56] in 2nd group and 1,87 U/ml [1,73; 2,31] in the control group. One of T1DM patients with the high titer of LACAb 8,89 U/ml also had celiac disease, another T1DM patient with the titer 6,64 U/ml had rheumatoid arthritis as well. No difference was found between 1st and 2nd groups ( $p = 0.11$ ). LACAb titer was significantly higher in patients with the duration of diabetes less than 5 years compared to other groups 4,02 [2,36; 8,56] ( $p < 0.05$ ). We also observed a trend to LACAb titer reduction with increasing of disease duration.

**Conclusion:** The study showed a high frequency of LACAb occurrence in patients with T1DM. These findings suggest the involvement of autoimmunity against the exocrine part of pancreas as well as against the endocrine part in some type 1 diabetic patients. These data may indicate the increased risk of chronic pancreatitis development in LACAb positive T1DM patients.

466

**Antibodies to beta cells antigens in cases of glucose metabolism disorders and obesity in children**

O.A. Budreiko, N.V. Shlyachova, L.D. Nikitina, S.O. Chumak, N.V. Filipova;  
State Institution "Institute of Children and Adolescents Health Care of National Academy of Medical Sciences", Kharkiv, Ukraine.

**Background and aims:** The presence of organ-specific antibodies against  $\beta$ -cells of pancreas is considered an important marker of their autoimmune destruction and predicts development of type 1 diabetes mellitus (DM1). However, the role of separate diabetogenic antibodies remains to date unclear in pathogenesis of type 2 diabetes mellitus (DM2) and other disorders of carbohydrate metabolism, especially in children and young people. Therefore, this study was aimed to investigate the production of antibodies against the most important diagnostic markers of autoimmune destruction of islet cells - antibodies against GAD (GADA) and tyrosine phosphatase autoantibodies (IA-2A), in children with impaired glucose metabolism (DM2, impaired glucose tolerance (IGT), impaired fasting glucose (IFG)), and insulin resistance (IR) against the background of obesity.

**Materials and methods:** A total of 125 children (65 boys and 60 girls) of age 7 to 18 years with impaired glucose metabolism (with IGT -  $n = 19$ , IFG -  $n = 20$ , with DM2 -  $n = 14$  and DM1 -  $n = 28$ ), obesity ( $n = 44$ , including those with IR -  $n = 22$ , and without IR -  $n = 22$ ) as well as 36 of their healthy peers were examined. For identification of carbohydrate metabolism disorders a OGTT was carried out according to WHO criteria, to determine the presence of glycemia, glycosuria, HbA1c. Determined was a level in serum of cytokines TNF $\alpha$ , IL1 $\beta$ , IL2, IL6 and IL10 (ELISA), of GADA and IA-2A, of C-peptide, of immunoreactive insulin (RIA). The study has been reviewed by the Local Ethics Committee and has been performed in accordance with the ethical standards laid down in the Helsinki Declaration.

**Results:** It was determined that some children with obesity (11%) display increased levels of GADA and/or IA-2A, but average indexes for the group

were  $1,02 \pm 0,10$  U/ml and  $0,78 \pm 0,06$  U/ml and did not differ significantly from those in the control group ( $0,80 \pm 0,09$  U/ml and  $0,45 \pm 0,02$  U/ml). In patients with DM1 levels of GADA ( $14,80 \pm 1,10$  U/ml) and IA-2A ( $11,36 \pm 0,87$  U/ml) were significantly increased ( $p < 0.01$ ), while in patients with DM2 they were increased as well, but to a lesser extent ( $8,77 \pm 0,19$  U/ml and  $7,43 \pm 0,62$  U/ml, respectively,  $p < 0.05$ ). Among other disorders of carbohydrate metabolism only patients with IGT displayed increased level of GADA ( $5,35 \pm 0,24$  U/ml,  $p < 0.05$ ) but not against IA-2A ( $0,84 \pm 0,11$  U/ml). Connection of insulinosecretion reduction with presence of antibodies against  $\beta$ -cells of pancreas has been proved only for patients with DM1, while children with obesity did not display differences in level of C-peptide dependent on presence of GADA and IA-2A. For patients with obesity the correlation is established between the level of antibodies against  $\beta$ -cells in blood and the content of separate cytokines: between GADA and IL6 ( $r = 0,36$ ,  $p < 0.05$ ), GADA and TNF $\alpha$  ( $r = 0,45$ ,  $p < 0.05$ ), GADA and IL-10 ( $r = 0,34$ ,  $p < 0.05$ ), IA-2A and IL6 ( $r = 0,29$ ,  $p < 0.05$ ).

**Conclusion:** A number of children with obesity, DM2 and IGT displayed signs of autoimmune aggression against pancreatic islets with involvement of cytokines in the pathological process. Although identified disorders were not accompanied by a pathological reduction of insulinosecretion in patients, the potential risk of severe carbohydrate metabolism disorders requires further study of the production of antibodies against antigens of  $\beta$ -cells in children with obesity, especially in combination with carbohydrate metabolism disorders.

467

**Coxsackievirus initiates strong immune response and death of beta cells**

E. Domsgen<sup>1</sup>, F. Paroni<sup>1</sup>, J. Kerr-Conte<sup>2</sup>, A. Dotzauer<sup>1</sup>, K. Maedler<sup>1</sup>;

<sup>1</sup>Centre for Biomolecular Interactions, University of Bremen, Germany,

<sup>2</sup>University of Lille, France.

**Background and aims:** Group B Coxsackievirus (CVB) infection of  $\beta$ -cells is associated with diabetes and induction of inflammation mediates  $\beta$ -cell destruction. Intra-islet viral particles were detected in patients with T1DM and T2DM, but the mechanisms of a correlation between virus infection and diabetes progression are poorly understood. In this study we asked the question, to which pattern recognition receptors (PRR) viral RNAs bind and which intracellular signals are induced to stimulate an inflammatory stage in human islets.

**Materials and methods:** Isolated human islets were infected with two different CVB serotypes, CVB3 and 4. Replication of CVB was confirmed by immunostaining of viral protein 1 (VP1) and titration of islet lysate. CXCL10 secretion from the islets was measured by ELISA; CXCL10, IFN $\beta$ , IFN $\gamma$ , IL-1 $\beta$ , TNF $\alpha$ , IL-6, MCP1, IL-8, RIG-I, TLR3, TLR7, MDA-5 and PKR mRNA production by quantitative RT-PCR and  $\beta$ -cell apoptosis by double-staining for the TUNEL assay and insulin. Islet protein expression and phosphorylation were analyzed by western blot. Binding of CVB ssRNA and dsRNA was investigated by immunoprecipitation-RT-PCR coupled assays.

**Results:** Isolated human islets were permissive for CVB, and upon infection,  $\beta$ -cell apoptosis was 7-fold increased. GSIS of infected islets showed impaired function at 72h post infection. The chemokine CXCL10, a known  $\beta$ -cell apoptosis inducer, was secreted upon infection, indicating induction of immune response. Immunoprecipitation-RT-PCR coupled assays demonstrated that viral CVB3 and 4 ssRNA bound to TLR3 and CVB4 ssRNA additionally to TLR7. Both CVB 3 and 4 dsRNA bound to protein kinase R (PKR), induced its phosphorylation. Lack of PKR in  $\beta$ -cells further increased apoptosis, suggesting that PKR protects the  $\beta$ -cell from virus effects. In contrast, retinoic acid inducible gene I (RIG-I) and Melanoma differentiation associated protein 5 (MDA-5) binding to CVB-dsRNA occurred secondarily in a later stage during infection. TLR3 sensing resulted in increased mRNA levels of the cytokines/chemokines CXCL10, IFN $\beta$ , IL-1 $\beta$ , TNF $\alpha$ , IL-6, MCP1 and IL-8 in the infected islets, while IFN $\gamma$  remained unchanged. CXCL10 was the highest induced factor (15-fold increase in mRNA,  $p < 0.001$ ), compared to uninfected islets. mRNA levels of TLR3, RIG-I, MDA5 and PKR were increased during infection. Immunostaining of TLR3 showed colocalisation with  $\beta$ -cells, while TLR7 was mainly expressed in  $\alpha$ -cells.  $\alpha$ - and  $\beta$ -cell specific co-staining with VP1 showed that CVB3 and 4 infected mainly the  $\beta$ -cells (60%  $\beta$ -cells were VP1 positive). While CVB3 infection led to VP1 expression in 5% of the  $\alpha$ -cells, CB4 showed 10% VP1/glucagon co-staining, suggesting an additional tropism of CVB4 for  $\alpha$ -cells. This did not result in differences in  $\alpha$ -cell survival. Both viruses induced apoptosis in  $\beta$ -cells.

**Conclusion:** Our data show that CBV infections have a direct deleterious effect on  $\beta$ -cell survival, resulting from virus-induced apoptosis and activation

of pro-inflammatory cytokines and chemokines as well as upregulation of PRR. CVB3 and 4 showed high tropism to TLR3 expressing  $\beta$ -cells, but CVB4 also bound to TLR7 expressed in  $\alpha$ -cells showing that virus sensing of TLR3 and 7 is strain and cell type specific. With our present data we provide novel targets towards protecting the  $\beta$ -cell during virus infection.

Supported by: DFG

## 468

### Beta cell expression of enteroviral receptor CAR and islet inflammation in human type 1 diabetes

I. Spagnuolo<sup>1,2</sup>, A. Patti<sup>1,2</sup>, G. Sebastiani<sup>1,2</sup>, F.A. Grieco<sup>1,2</sup>, F. Dotta<sup>1,2</sup>;

<sup>1</sup>Diabetes Unit, Department of Internal Medicine, Endocrine and Metabolic Sciences and Biochemistry, University of Siena, <sup>2</sup>Fondazione Umberto Di Mario ONLUS - Toscana Life Science, Siena, Italy.

**Background and aims:** Type 1 diabetes mellitus (T1D) is a chronic autoimmune disease in which pancreatic beta cells are selectively destroyed by an autoimmune process. The onset is caused by the interaction of genetic, immunological and environmental factors. Among the environmental factors potentially involved in the T1D etiology, viral infections play a key role. Previous studies have demonstrated signs of enteroviral infection in pancreatic islets, and most intriguingly in beta cells, from recent-onset T1D patients. Moreover it has been suggested that Coxsackie B4 virus (CVB4) directly trigger beta-cell destruction, suggesting a link between enteroviral infections and T1D. The susceptibility to viral infections is caused by viral variant and by the nature of infection, while the specific tropism of viruses is modulated by the local expression of cellular receptors, such as hCAR (coxsackievirus and adenovirus receptor). We here aimed at characterizing islet inflammation and hCAR islet expression in human type 1 diabetes.

**Materials and methods:** We studied pancreatic specimens obtained from 4 recent onset T1D, from 2 long standing (disease duration: 7 and 14 years) T1D and from 10 non-diabetic organ donors. Formalin-fixed and paraffin embedded pancreatic sections were used in immunohistochemical experiments for the presence of proinflammatory molecules (CXCL10, CCL2, Fas) as well as of viral receptor hCAR. In addition, double immunofluorescence with confocal microscopy analysis was utilized for the identification islet cell subset(s) expressing the molecule of interest (i.e. alpha-, beta- and delta cells identified by anti-glucagon, anti-insulin and anti-somatostatin antibodies respectively).

**Results:** In T1D pancreata, insulitis was present in 44% of islets and was characterized by CD3 expressing T-cells and natural killer cells. Ongoing islet inflammation was detected in all T1D cases, but not in control donors, with in situ detection of CXCL10, Fas and of CCL2; interestingly, this latter chemokine showed a remarkable beta-cell specific expression. Viral receptor hCAR was expressed both in T1D and in control donors mainly in islet cells and, of note, almost exclusively at beta cell level, suggesting the existence of a differential distribution of enterovirus receptors among islet cell subsets, possibly influencing the susceptibility of these cells to enteroviral infection. Intriguingly, the only rare alpha cells expressing hCAR were single cells located outside islet structures and sparse throughout the exocrine tissue.

**Conclusion:** In conclusion, we have shown that islet inflammation can be detected at disease onset, persists years after T1D diagnosis and is characterized by proinflammatory molecules and by a beta cell specific expression chemokine CCL2. In addition, we uncovered the beta-cell expression of enteroviral receptor hCAR, which may represent a mechanism responsible for the beta cell tropism observed in the case of some enteroviruses.

Supported by: EU - FP7 grant PEVNET

## 469

### ISG15 detected in beta cells of fulminant type 1 diabetes indicates anti-apoptotic effect in MIN6 cells

A. Yoshikawa, A. Imagawa, S. Nakata, S. Uno, K. Fukui, Y. Kuroda, Y. Miyata, Y. Sato, T.-A. Matsuoka, H. Iwahashi, I. Shimomura;  
Metabolic Medicine, Graduate School of Medicine, Osaka University, Suita, Japan.

**Background and aims:** Involvement of viral infections has been suggested in the pathogenesis of fulminant type 1 diabetes. We focused on ISG15 located in the downstream of type I interferon signaling and investigated its expression in pancreatic autopsy specimens and its role in mouse beta cell line MIN6.

**Materials and methods:** We examined pancreas autopsy specimens from three patients with fulminant type 1 diabetes who had died just after the onset of overt diabetes. To visualize the pancreatic beta cells, alpha cells and ISG15, sections were stained using the indirect immunohistochemical technique. Recombinant adenoviruses expressing ISG15 and short hairpin RNA against ISG15 were prepared using the pAdEasy system. The expression of ISG15 mRNA was assessed by real-time quantitative RT-PCR and the production of ISG15 protein was detected by western blots in MIN6 cells. To assess the cell apoptosis, Caspase 3/7 activity assay was performed with Caspase-Glo 3/7 assay Systems.

**Results:** Immunohistochemical analysis revealed that the expression of ISG15 was detected in islets of all three patients and also in exocrine pancreas of one patient with fulminant type 1 diabetes, but neither in islets nor in exocrine pancreas of all three control subjects. By double staining for ISG15 and islet hormones, ISG15 was expressed in insulin-positive cells but not in glucagon-positive cells. Neither ISG15 mRNA nor protein was detected in MIN6 cells without treatment of IFN alpha. IFN alpha upregulated ISG15 mRNA and protein production in MIN6 cells at dose dependent manner. Expression of ISG15 mRNA and protein production was increased by treatment of Ad-ISG15, but not by treatment of Ad-GFP. After incubation with Ad-GFP, then exposure to IFN gamma plus IL-1beta result in an approximate 3.3-fold increase in Caspase 3/7 activity. Treatment with Ad-ISG15 in addition to IFN gamma and IL-1beta decreased caspase 3/7 activity by 36.4% ( $P < 0.0001$ ). After incubation with Ad-GFP, then exposure to TNF alpha result in an approximate 3.0-fold increase in Caspase 3/7 activity. Treatment with Ad-ISG15 in addition to TNF alpha decreased caspase 3/7 activity by 34.4% ( $P = 0.0004$ ). On the other hand, expression of ISG15 mRNA and protein production was decreased by treatment of Ad-shISG15. After incubation with Ad-GFP, then exposure to IFN gamma and IL-1beta and IFN alpha result in an approximate 2.2-fold increase in Caspase 3/7 activity. Treatment with Ad-shISG15 increased caspase 3/7 activity by 32.3% ( $P = 0.0002$ ). After incubation with Ad-GFP, then exposure to TNF alpha and IFN alpha result in an approximate 3.8-fold increase in Caspase 3/7 activity. Treatment with Ad-shISG15 increased caspase 3/7 activity by 21.4%, but not significant ( $P = 0.46$ ). Those results indicate that apoptotic effect of inflammatory cytokines decreased by the addition of and increased by the deletion of ISG15.

**Conclusion:** ISG15 was expressed in pancreatic beta cells in fulminant type 1 diabetes and anti-apoptotic effect of ISG 15 was observed in MIN6 cells, suggesting a possible therapeutic approach by the induction of ISG15 in fulminant type 1 diabetes.

Supported by: KAKENHI (JSPS, MHLW)

## 470

### St. John's Wort and hyperforin inhibit multiple phosphorylation steps of cytokine signalling in beta cells and modulate functional, inflammatory and apoptotic gene expression

P. Masiello<sup>1</sup>, P. Beffy<sup>2</sup>, M. Novelli<sup>1</sup>, S. Porozov<sup>1</sup>, A. Sgarbossa<sup>3</sup>, M. Masini<sup>1</sup>, V. De Tata<sup>1</sup>, L. Martino<sup>1</sup>, A. Pippa<sup>4</sup>, M. Menegazzi<sup>3</sup>;

<sup>1</sup>Dip. Patologia Sperimentale, University of Pisa, <sup>2</sup>Istituto di Fisiologia Clinica, CNR, Pisa, <sup>3</sup>Dip. Scienze della Vita e della Riproduzione, University of Verona, <sup>4</sup>Sant'Anna School of Advanced Studies, Pisa, Italy.

**Background and aims:** Cytokines released by mononuclear cells infiltrating the islets of Langerhans during an autoimmune attack are considered responsible for the  $\beta$ -cell destruction leading to type 1 diabetes, through STAT-1 and NF- $\kappa$ B activation and consequent expression of deleterious target genes. We have previously shown that the extract of Hypericum perforatum (St. John's wort, SJW) and its phloroglucinol component hyperforin (HPF), are potent inhibitors of cytokine-induced STAT-1 and NF- $\kappa$ B activation in  $\beta$ -cells and prevent dysfunction and apoptosis in INS-1E  $\beta$ -cell line, as well as in rat and human islets. Aims of this study were: a) to further clarify the mechanism of the regulatory activity of SJW and HPF on cytokine signaling pathways in INS-1E cells; b) to assess their ability to counteract the cytokine-driven changes in the expression of STAT-1 and NF- $\kappa$ B target genes involved in  $\beta$ -cell function, inflammatory response and apoptosis regulation.

**Materials and methods:** INS-1E cells, exposed to mixtures of IFN- $\gamma$ , IL-1 $\beta$  and TNF- $\alpha$  for various time periods with/without SJW extract (1-2  $\mu$ g/ml) or HPF (1-2  $\mu$ mol/l), were used for RT-qPCR gene expression analysis and assessment of the phosphorylation state of various components of STAT-1, NF- $\kappa$ B and MAPK pathways by western blotting. STAT-1 and NF- $\kappa$ B activation was also evaluated by EMSA on nuclear extracts.

**Results:** Cytokine-induced STAT-1 phosphorylation in both tyrosine and serine residues (2-3-fold increase vs. controls) was significantly hindered

by SJW or HPF in a dose-dependent manner. These compounds prevented NF- $\kappa$ B activation by suppressing phosphorylation of the p65 subunit and the inhibitory subunit I $\kappa$ B activating kinase (IKK). Furthermore, MAPK pathway was also modulated by the vegetal compounds through dose-dependent partial or total restriction of ERK1/2, p38 MAPK and JNK cytokine-induced phosphorylations. Inhibition of DNA binding of STAT-1 and NF- $\kappa$ B in the presence of SJW or HPF was confirmed by EMSA. Expressions of a number of  $\beta$ -cell functional genes, such as PDX-1, GLUT-2 and FOXO1, were down-regulated by 60–80% ( $p < 0.05$  vs. controls) upon cytokine treatment and restored in the presence of SJW and HPF, while expressions of insulin, glucokinase, MAFA, PAX-6 genes were less affected. A remarkable induction ( $>10$ -fold vs. controls) of iNOS and other pro-inflammatory genes (CXCL9, CXCL10, COX-2, ICAM-1, MHC-2 trans-activator) was elicited by cytokines in INS-1E cells and significantly reduced or totally abolished by vegetal compounds in a dose-dependent fashion. SJW and HPF were able to partially correct the cytokine-induced unbalance between anti- and pro-apoptotic factors, mainly by preventing down-regulation of anti-apoptotic members of BCL-2 family.

**Conclusion:** We provide evidence that SJW extract and hyperforin exert their protective effects against cytokine-induced  $\beta$ -cell damage by inhibiting multiple phosphorylation steps along the STAT-1, NF- $\kappa$ B and MAPK signaling pathways and modulating target gene expression. Thus, SJW components represent novel promising pharmacological tools for prevention or limitation of  $\beta$ -cell loss in type 1 diabetes.

*Supported by: Telethon-Italy/IDRF (grant n. GJT08016)*

## 471

### IFN- $\lambda$ elicits an antiviral state and protects human islets from coxsackievirus infection

M. Flodström-Tullberg, K. Lind;

Center for Infectious Medicine, Dept. of Medicine HS, Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** Infections with enteroviruses such as Coxsackie B viruses (CVB) have been associated with the development of type 1 diabetes (T1D). CVBs infect human islets in vitro and enterovirus has been found in islet remnants of T1D patients. How the host antiviral response regulates human islet permissiveness to infection is not fully understood. We have previously shown that type I and II interferons (IFNs) induce an antiviral state in human islets, lowering the islets' permissiveness to CVB infection. Type III IFNs (IFN $\lambda$ 1/IL29, IFN $\lambda$ 2/IL28A, and IFN $\lambda$ 3/IL28B) belong to a recently identified novel group of IFNs. They are produced in response to some viruses and activate antiviral defence in responsive cells. IFN $\lambda$ s bind to a receptor complex consisting of IL-10R2 and IL-28R $\alpha$ . The former receptor unit is ubiquitously expressed, while the latter shows a restricted tissue distribution. We recently observed that CVB infected human islets express IFN $\lambda$ s (unpublished observation). However, whether human islets respond to IFN $\lambda$ s remains unknown. Moreover, whether IFN $\lambda$ s prevent CVB replication has not been studied in any type of cell. Here, we investigated whether human islets express the receptor for IFN $\lambda$ s and respond to IFN $\lambda$  stimulation. Importantly, we also determined whether IFN $\lambda$ s protect human islets from CVB infection.

**Materials and methods:** The expression of IFN $\lambda$  receptor subunits and the response to IFN $\lambda$  exposure were analysed in human islets obtained from The Nordic Network for Clinical Islet Transplantation using Real-time (RT)-PCR or PCRarray (Qiagen). RNA from human peripheral blood mononuclear cells (PBMC) and hepatocyte cell lines were used as positive controls for IFN $\lambda$  receptor expression. In separate experiments, human islets were pre-treated with IFN $\lambda$ 1 (100 ng/ul) or IFN $\lambda$ 2 (100 ng/ul) for 24h and then infected with  $4 \times 10^4$  PFU/ml of Coxsackievirus B3 (CVB3). Viral titers in supernatants were assessed using a standard plaque assay technique.

**Results:** We demonstrate that human islets express mRNA encoding the IFN $\lambda$  receptor subunits IL-10R2 and IL-28R $\alpha$  at similar levels as human PBMCs. We also show that IFN $\lambda$ s induce the mRNA expression of several genes involved in the antiviral state (e.g. MxA, OAS) as well as pattern recognition receptors (e.g. IFIH1, RIG-I and TLR3) in human islets. Finally, we demonstrate that CVB3 replicates to significantly lower titers in IFN $\lambda$  treated human islets compared to untreated islets ( $p < 0.01$  vs. infected islets that were mock-treated).

**Conclusion:** Our results clearly suggest that human islets express IFN $\lambda$  receptors and respond to IFN $\lambda$  treatment by upregulating genes involved in antiviral defense and recognition of RNA viruses. By showing that CVB3 replication is perturbed in IFN $\lambda$  treated human islets we for the first time demonstrate that IFN $\lambda$  limits CVB replication in primary human cells. Col-

lectively, our results strongly support an important role for IFN $\lambda$ s in regulating human islet permissiveness to CVB infection. Thus, IFN $\lambda$ s may contribute to reduced tissue damage and promote host and beta cell survival upon an enterovirus infection.

*Supported by: Karolinska Institutet, Sweden, the European Union (FP7)*



## PS 025 Clinical immunology: immune markers in type 1 diabetes

472

### Cytokine-producing function of peripheral blood cells in the patients with type 1 diabetes mellitus

E.A. Repina<sup>1</sup>, E.N. Stepanova<sup>2</sup>;<sup>1</sup>Laboratory of Immunology and Genetics, Endocrinological Research Centre, Moscow, <sup>2</sup>Chair of Immunology, Russian Medical Academy of Postgraduate Education, Moscow, Russian Federation.

**Background and aims:** To investigate an influence of polyclonal activator - phytohemagglutinin and insulin upon cytokine-producing function of peripheral blood cells in the patients with type 1 diabetes mellitus.

**Materials and methods:** 25 type 1 diabetes mellitus patients were examined. They were divided into the following two groups: patients with duration of type 1 diabetes mellitus under 10 years (group 1) and patients with duration of type 1 diabetes mellitus more than 10 years (group 2). Mononuclear leukocytes were isolated by centrifugation in the ficoll-verographin density gradient. The cells thus obtained were resuspended in the complete nutrient medium reducing their concentration to  $2.0 \times 10^6$ /ml. Phytohemagglutinin (PHA-P, Sigma) (10 mcg/ml) and insulin (insulin human, Sigma) (10 mcg/ml) were added to the samples to stimulate mononuclear leukocytes; cell suspensions were further incubated for 36 hours. Initial, PHA-induced and insulin-induced levels of interleukin-1 (IL-1), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, gamma interferon (IFN- $\gamma$ ) and tumor necrosis factor alpha (TNF- $\alpha$ ) in supernatants of cell cultures were measured by solid phase immunoassay at 450 nm. Results were compared with clinical characteristics of the patients. There were 3 males and 5 females in group 1, 5 males and 12 females in group 2. Median age, diabetes mellitus duration, HbA1c in group 1 and group 2 were respectively 27 years, 3 years, 7.2 % and 38 years, 23 years, 10.3 %. Intensities of microvascular complications (diabetic retinopathy and diabetic nephropathy) were higher in the group 2. 3 patients (37.5 %) from group 1 had diabetic retinopathy on the basic stage and 8 patients (47 %) from group 2 had diabetic retinopathy on the last stage. Only 1 patient from group 1 had diabetic nephropathy on microalbuminuria stage and 7 patients from group 2 (41%) had diabetic nephropathy on proteinuria stage and on the stage of renal failor.

**Results:** Basal and PHA-induced production of IL-2, IL-4, IL-10, IL-12 were significantly higher in group 1 (0.83/7.8; 3.7/20.3; 7.35/58.7; 0.8/5.3) in comparison with group 2 (0.35/6.3; 2.3/13.5; 3.6/27.8; 0.3/1.7) ( $p=0.026/p=0.03$ ;  $p=0.05/p=0.03$ ;  $p=0.01/p=0.027$  and  $p=0.019/p=0.001$ ). At the same time, basal and PHA-induced levels of IL-1, IFN- $\gamma$  and TNF- $\alpha$  were significantly higher in group 2. These markers had significant positive correlation with microvascular complications (diabetic retinopathy and diabetic nephropathy) ( $R=0.78$   $p=0.043$ ;  $R=0.7$   $p=0.05$ ;  $R=0.67$   $p=0.04$  and  $R=0.72$   $p=0.043$ ;  $R=0.64$   $p=0.032$ ;  $R=0.64$   $p=0.05$ ).

**Conclusion:** The cytokine production ratio of stimulated versus resting cells may be used as a reliable predictor of disease severity in type 1 diabetic patients and microvascular complications in type 1 diabetes mellitus.

473

### CD4+ T cell proliferation responses after wheat polypeptide stimulation in children at different stages of type 1 diabetes autoimmunity

S. Hamari<sup>1</sup>, T. Kirveskoski<sup>1</sup>, V. Glumoff<sup>2</sup>, O. Simell<sup>3</sup>, M. Knip<sup>4</sup>, J. Ilonen<sup>5</sup>, R. Veijola<sup>1</sup>;<sup>1</sup>Department of Pediatrics, University of Oulu, <sup>2</sup>Department of Medical Microbiology, University of Oulu, <sup>3</sup>Department of Pediatrics, University of Turku, <sup>4</sup>Institute of Clinical Medicine, University of Helsinki, <sup>5</sup>Immunogenetics Laboratory, University of Turku, Finland.

**Background and aims:** Recent evidence suggests that immune reactivity of CD4+ lymphocytes is increased after *in vitro* wheat protein stimulation in patients with type 1 diabetes (T1D) of several years' duration. The aim of this study was to evaluate if there is any difference in T-cell responses after *in vitro* stimulation with different wheat polypeptides in recently seroconverted children and in children with newly-diagnosed type 1 diabetes compared to healthy controls.

**Materials and methods:** Peripheral blood mononuclear cells (PBMCs) were isolated 3-6 months after seroconversion for beta-cell specific autoantibodies from 34 subjects aged 1-15 years and 30 control subjects. In the second study

group the PBMCs were isolated 5-12 days after the diagnosis of T1D from 52 subjects aged 1-15 years and 51 control subjects. The control subjects of both groups were age-, sex- and sampling date matched healthy and autoantibody negative children carrying HLA-DQB1 risk associated genotypes. All study subjects were negative for tissue transglutaminase antibodies. Carboxy-fluorescein succinimidyl ester (CFSE) based T-cell proliferation assay was performed from freshly ( $< 8$  hours) isolated PBMCs and the proliferation responses were analyzed after 7 days *in vitro* antigen (tetanus toxoid, gliadin, gluten, whole wheat) stimulation. The positive proliferation response was defined as a cell division index (CDI) of at least 2.0 and the relative amount of proliferated CD4+ T-cells at least 0.5% from the parent gate. Only subjects with a positive tetanus toxoid response were included in the analysis.

**Results:** The frequencies of positive *in vitro* T cell responses for all three wheat proteins were similar between recently seroconverted children and controls (gliadin 73.5% vs. 73.3%;  $p=1.000$ , gluten 85.3% vs. 83.3%;  $p=1.000$  and whole wheat 23.5% vs. 26.7%;  $p=0.781$ ). In children with newly-diagnosed T1D the proportion of positive responses after *in vitro* gliadin stimulation was clearly decreased when compared to healthy controls (48.1% vs. 76.5%;  $p=0.004$ ), and a similar trend was seen for gluten and whole wheat responses (gluten 71.2% vs. 84.3%;  $p=0.155$  and whole wheat 15.4% vs. 25.5%;  $p=0.230$ ). No statistically significant differences were observed in the strength of positive responses (median CDI values) between the study and control groups.

**Conclusion:** Positive T-cell immune responses for wheat-based antigens are common in children. The frequencies of positive proliferation responses for gliadin, gluten and whole wheat were similar between children who had recently seroconverted positive for beta-cell specific autoantibodies and their controls. Decreased frequency of positive *in vitro* gliadin responses were observed in children with newly-diagnosed type 1 diabetes.

*Supported by: The Alma and K. A. Snellman Foundation, FPR, The Diabetes Research Foundation*

474

### Study of the insulin receptor (CD220) expression in peripheral immune cells of patients with type 1 diabetes

S.A. Paschou<sup>1</sup>, A. Petsiou<sup>2</sup>, G. Vartholomatos<sup>2</sup>, N. Kolaitis<sup>2</sup>, E. Giotaki<sup>3</sup>, A. Tsatsoulis<sup>1</sup>, G.K. Papadopoulos<sup>4</sup>;<sup>1</sup>Department of Endocrinology, Medical School, University of Ioannina,<sup>2</sup>Unit of Molecular Biology, Laboratory of Haematology, University Hospitalof Ioannina, <sup>3</sup>Department of Nursing, Epirus Institute of Technology,Ioannina, <sup>4</sup>Laboratory of Biochemistry and Biophysics, Epirus Institute of Technology, Arta, Greece.

**Background and aims:** Insulin receptors are essentially ubiquitous, yet scant attention has been paid to the possible actions of insulin on the immune system either in health or in an insulinopenic state such as type 1 diabetes. The aim of our study was to investigate the membrane expression of insulin receptor (CD220) in three very important categories of peripheral immune cells, T effector cells (Teff, CD4<sup>+</sup>CD25<sup>+</sup>), T regulatory cells (Tregs, CD4<sup>dim</sup>CD25<sup>high</sup>FOXP3<sup>high</sup>CD127<sup>low</sup>) and monocytes, of patients with type 1 diabetes in comparison with healthy controls.

**Materials and methods:** Peripheral blood from 39 patients with type 1 diabetes (13 newly-diagnosed ones (nd: 9M/4F, ages  $12.5 \pm 9.4$  years) and 26 long standing ones (ls: 12 M/14F, ages  $26.7 \pm 9.2$  years, with mean disease duration of  $11.6 \pm 7.4$  years), and 32 healthy controls (c) with no first or second degree relatives suffering from any autoimmune disease (13M/19F, ages  $25.3 \pm 11$  years) was analysed by triple colour flow cytometry for the membrane expression of insulin receptor (CD220) in Teff, Tregs and monocytes (without any Ficoll-paque mononuclear cell isolation).

**Results:** Insulin receptor is clearly expressed in the membrane of Teff (%  $1.65 \pm 4.38$ , MFI (Mean Fluorescence Intensity - number of molecules per cell)  $62.63 \pm 81.32$ ), Tregs (%  $9.30 \pm 25.15$ , MFI  $81.87 \pm 169.35$ ) and monocytes (%  $17.56 \pm 28.14$ , MFI  $486.27 \pm 1,354.80$ ) of healthy controls with a clear trend of higher levels in monocytes and Tregs than Teff ( $p = 0.006$  &  $p = 0.093$ ). The expression of insulin receptor in patients with type 1 diabetes in Teff (nd: %  $1.95 \pm 5.05$ , MFI  $96.95 \pm 180.34$ , ls: %  $0.37 \pm 0.64$ , MFI  $68.63 \pm 73.85$ ) and Tregs (nd: %  $3.88 \pm 7.90$ , MFI  $51.23 \pm 95.41$ , ls: %  $1.77 \pm 2.58$ , MFI  $185.7 \pm 455.24$ ) tends to be lower than in healthy controls, but without statistical significance (in all comparisons  $p > 0.05$ ). In the case of monocytes, the difference in the frequency of insulin receptor between controls and diabetic patients is substantial and statistically significant (nd: %  $2.17 \pm 4.94$ , MFI  $316.23 \pm 460.27$ , ls: %  $0.24 \pm 0.37$ , MFI  $507.31 \pm 1,651.11$ ,  $p = 0.015$  &  $p = 0.006$ ), altering the pattern of its expression (Monocytes > Tregs > Teff) that we meet in healthy controls.

**Conclusion:** The lower frequency of insulin receptor expression in monocytes of patients with type 1 diabetes may affect the functionality of these important immune cells, especially in combination with the low levels of insulin in the immediate prediabetes phase, and can be one of the reasons for impaired suppression of the immune response in these patients.

*Supported by: Archimedes I to GKP, 3rd CSF (75% EU funds, 25% Hellenic state funds)*

## 475

### CXCR3<sup>+</sup>, CCR4<sup>+</sup> T memory cells and chemokine levels: comparison between recent onset type 1 diabetics and nondiabetic first degree relatives

T. Milicic<sup>1</sup>, N.M. Lalic<sup>1</sup>, A. Jotic<sup>1</sup>, I. Markovic<sup>2</sup>, M. Zamaklar<sup>1</sup>, K. Lalic<sup>1</sup>, L. Lukic<sup>1</sup>, N. Rajkovic<sup>1</sup>, M. Macetic<sup>1</sup>, J. Seferovic<sup>1</sup>;

<sup>1</sup>Clinical Center of Serbia, Clinic for Endocrinology, <sup>2</sup>Institute for Biochemistry, Belgrade, Serbia.

**Background and aims:** It has been previously suggested that chemokine receptors CXCR3 and CCR4, and its ligands, interferon- $\gamma$  inducible chemokine (IP-10) and thymus- and activation-regulated chemokine (TARC), are associated with Th1 and Th2 memory cell subsets respectively, and have important role in the initial phase of Type 1 diabetes (T1D). However, the changes in CXCR3<sup>+</sup> and CCR4<sup>+</sup> subsets of the T memory cells as well as in chemokine levels, IP-10 and TARC, in recent onset T1D and their nondiabetic first-degree relatives (FDRs), have not yet been elucidated. Therefore, the aim of this study was to analyze: (a) the percentage of CXCR3<sup>+</sup> (Th1 associated) and CCR4<sup>+</sup> (Th2 associated) subsets of T memory cells (b) chemokine levels IP-10 (Th1 associated) and TARC (Th2 associated), in peripheral blood in 27 recent-onset T1D patients in insulin-requiring state (IRS) at the onset (group A), 16 T1D patients in the state of clinical remission (CR) (group B), 43 nondiabetic FDRs (group C), as well as in 22 healthy, age-matched control subjects (group D).

**Materials and methods:** T1D was diagnosed in accordance to WHO criteria. The CR was defined as optimal metabolic control without insulin lasting >30 days. The percentages of CXCR3<sup>+</sup> and CCR4<sup>+</sup> T memory cell subsets were analyzed in peripheral blood by using four-color immunofluorescence staining and flowcytometry. IP-10 and TARC were determined by ELISA.

**Results:** We found that there was no difference among the groups concerning the percentage of T memory cells (A: 27.33 $\pm$ 7.54 vs B: 24.88 $\pm$ 6.23 vs C: 25.65 $\pm$ 6.43 vs D: 27.22 $\pm$ 5.52 %, A vs B vs C vs D, p=NS). However, when the percentage of CXCR3<sup>+</sup> T memory cells was analyzed, we found that in groups A and B it was significantly lower than in groups C and D (A: 40.10 $\pm$ 10.46; B: 42.04 $\pm$ 11.22; C: 53.98 $\pm$ 8.23; D: 53.12 $\pm$ 6.45 %; A vs C, D: p<0.001; B vs C, D: p<0.01), while there was no difference between groups C and D. Simultaneously, the percentage of CCR4<sup>+</sup> T memory cells was also found to be significantly lower in groups A and B than in groups C and D (A: 31.65 $\pm$ 9.69; B: 31.42 $\pm$ 8.23; C: 39.92 $\pm$ 9.24; D: 40.96 $\pm$ 7.29%; A vs C, D and B vs C, D p<0.01) also without difference between groups C and D. On the other hand, IP-10 and TARC levels were significantly higher in groups A and B than in groups C and D (A: 146.99 $\pm$ 68.58; 399.55 $\pm$ 322.56, B: 133.03 $\pm$ 63.19; 383.35 $\pm$ 120.97, C: 118.96 $\pm$ 87.42; 241.72 $\pm$ 164.61, D: 89.28 $\pm$ 19.89; 232.80 $\pm$ 89.34 pg/ml, respectively; A vs C, D and B vs C, D p<0.05), also without differences between groups C and D.

**Conclusion:** Our results demonstrated that the beginning of T1D was associated with the decrease in CXCR3<sup>+</sup> and CCR4<sup>+</sup> subsets of T memory cells, together with the increase in IP-10 and TARC levels, probably reflecting their homing and accumulation into inflamed pancreatic islets. However, these changes could not be identified in the nondiabetic FDRs, thus implying that the onset of T1D might be strongly influenced on the level of these subsets of T memory cells and associated chemokines.

## 476

### Can some differences be found in T regulatory cell count and priming in cord blood of children born to type 1 diabetic mothers?

K. Stechova<sup>1</sup>, T. Ulmannova<sup>1</sup>, D. Bartaskova<sup>2</sup>, J. Norkova<sup>1</sup>, I. Spalova<sup>3</sup>;

<sup>1</sup>Paediatric dpt., <sup>2</sup>Dpt. of Internal Medicine, <sup>3</sup>Dpt. of Gynaecology and Obstetrics, University Hospital Motol and 2nd Medical Faculty of Charles University, Prague, Czech Republic.

**Background and aims:** Type 1 diabetes (T1D) develops due to autoimmune pancreatic beta cells destruction. The effect of diabetes on overall pregnancy

outcome is well documented. However, much less is known about the effect of maternal autoimmune diabetes on the developing foetal immune system. The risk of T1D development in a child of T1D mother who was diagnosed prior pregnancy is lower than e.g. in a child of T1D father. This discrepancy supports the theory that there is some immunoregulatory influence on her baby. For this reason we decided to study T regulatory cells (Tregs) which are important in immunoregulation and for autoimmune diseases pathogenesis. Moreover human Tregs cord blood studies related to T1D are limited and there are only two studies from Scandinavia on this topic which suggested certain differences in Tregs in cord blood of children born to T1D mothers.

**Materials and methods:** Tregs defined as CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>-</sup> in cord blood (CB) samples were investigated in our study by flow cytometry (FACS) and 20 T1D mothers, 20 gestation diabetes mothers treated by insulin and 45 healthy controls were enrolled. Markers of naive resp. primed cells (CD45RA/RO) were analysed by FACS too. Inclusion criteria were gestational age at least 35+0 weeks, no major maternal or foetal complications (as preeclampsia, asphyxia etc.), maternal HbA1c in all trimesters below 7% according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

**Results:** We found only that CB from babies of T1D mothers contained 6.8 % Tregs/total T helper lymphocytes (Th) in comparison to 9.4 % in healthy controls (p = 0.045). Other differences were not significant and results were not influenced e.g. by delivery mode (Caesarean section vs physiological labour etc.).

**Conclusion:** If an establishment of specific immunological tolerance in foetus of T1D mother really exists, it seems to be rather due to other factors than just to changes in Tregs count (which was moreover found to be decreased in our study). We are currently focused on explanation why children of T1D mothers suffer from diabetes less frequently than other first degree relatives by using functional analysis of Tregs. Insights in to this phenomenon would be useful e.g. for construction of immunointervention therapy.

*Supported by: MZCR(00064203)*

## 477

### T regulatory cells in the peripheral blood of relatives of type 1 diabetes patients

A.N. Petsiou<sup>1</sup>, S.A. Paschou<sup>1</sup>, A. Karamoutsios<sup>2</sup>, G. Vartholomatos<sup>2</sup>, N. Kolaitis<sup>2</sup>, A. Tsatsoulis<sup>1</sup>, G.K. Papadopoulos<sup>3</sup>;

<sup>1</sup>Department of Endocrinology, Medical School, University of Ioannina,

<sup>2</sup>Unit of Molecular Biology, Laboratory of Haematology, University Hospital of Ioannina, <sup>3</sup>Laboratory of Biochemistry and Biophysics, Epirus Institute of Technology, Arta, Greece.

**Background and aims:** Type 1 diabetes mellitus (T1D) is believed to result from autoimmune destruction of the beta cells of the pancreas, but there is no unifying theory on the disease immunopathogenesis. An approach to immune abnormalities, could be done through the study of first degree relatives (FDR) of affected individuals, because even though T1D appears in “naive” families, FDR of T1D patients are considered a high-risk group for the disease. In order to investigate the mechanisms involved in the dysregulation of immune self tolerance, we studied T regulatory cells (Tregs) in families with an index case of T1D. Tregs are a subpopulation of CD4 T cells which appear to have the ability of contact-dependent suppression and regulation of the immune response. This subpopulation is characterised by high surface expression of the IL-2 receptor alpha chain (CD25) and down-regulation of the IL-7 $\alpha$  (CD127), which is correlated with high expression of the intracellular forkhead/winged-helix family transcription factor p3 (FoxP3), the most specific marker of Tregs. We had previously shown that Tregs are CD4<sup>dim</sup>CD25<sup>high</sup>CD127<sup>low</sup> T cells and are significantly reduced in newly-diagnosed and long-standing type 1 diabetes patients, compared to controls.

**Materials and methods:** Four colour flow cytometry (using the following markers: anti-CD4-PerCP, anti-CD25-APC, anti-CD127-PE, anti FoxP3-Alexa 488) was performed in peripheral blood of 38 FDR (17 siblings, age 22.7  $\pm$  10.4 years (mean  $\pm$  sd), 5 children aged 28.2  $\pm$  2 years, 9 mothers aged 36.4  $\pm$  7.8 years, and 7 fathers aged 46  $\pm$  7.8 years) and 45 healthy controls aged 31.6  $\pm$  14 years. We defined Tregs as the subpopulation of CD4<sup>dim</sup>CD25<sup>high</sup>CD127<sup>low</sup> T cells. In order to verify by independent means the estimated percentage of Tregs, we correlated this estimate with the percent of CD127<sup>low</sup> T cells (a hallmark of Tregs) within the top 7% of CD4<sup>+</sup>CD25<sup>high</sup> T cells (a percent that totally covers the putative Tregs population, active and resting), calling this the verifying population.

**Results:** In 12 controls tested, FoxP3 was expressed in 91.32  $\pm$  4.98 % of so-defined Tregs. The correlation between the percent of Tregs and the percent of CD127<sup>low</sup> T cells in the verifying population was very significant at the

$p < 0.01$  level ( $r = 0.899$ ). The Tregs population was not significantly lower ( $p < 0.05$ ) for any of the subgroups, as seen on the following table.

	TREGS % of CD4 T cells (mean $\pm$ sd)	$p$ (versus control)	VERIFYING POPULATION (mean $\pm$ sd)	$p$ (versus control)
Control	3.31 $\pm$ 0.87		67.78 $\pm$ 8.80	
Siblings	2.77 $\pm$ 1.12	0.087	62.97 $\pm$ 14.94	0.149
Mothers	3.2 $\pm$ 1.16	0.792	64.18 $\pm$ 13.67	0.467
Fathers	3.73 $\pm$ 1.57	0.512	70.73 $\pm$ 13.07	0.582
Children	2.8 $\pm$ 0.71	0.193	65.04 $\pm$ 8.46	0.525

**Conclusion:** By an independent means we have established that our method of estimating the percentage of Tregs is reliable and objective. The absence of significant differences in the percentage of Tregs in the various subpopulations of FDR does not rule out the importance of this subpopulation in T1D pathogenesis. An analysis of the phenotype of the Tregs in FDR is in progress. *Supported by: The Hellenic Endocrine Society by a small grant to AT, GKP and GV*

## 478

### Autoantibodies against the glutamate transporter GLT1, a specific beta cell plasma membrane protein, in the serum of type 1 diabetes mellitus subjects

C. Perego<sup>1</sup>, E.S. DI Cairano<sup>1</sup>, E. Fino<sup>1</sup>, C. Bazzini<sup>2</sup>, E. Bazzigaluppi<sup>3</sup>, A. Gastaldelli<sup>4</sup>, F. Bertuzzi<sup>5</sup>, A.M. Davalli<sup>6</sup>, F. Folli<sup>7</sup>;

<sup>1</sup>DISMAB Dept of Biol Science Applied to Biosystems, University of Milano, Italy, <sup>2</sup>Biomolecular Science and Biotechnology, University of Milano, Italy, <sup>3</sup>San Raffaele Hospital, Milano, Italy, <sup>4</sup>Institute of Clinical Physiology, CNR, Pisa, Italy, <sup>5</sup>Niguarda Cà Granda Hospital, Milano, Italy, <sup>6</sup>Internal Medicine, Diabetes & Endocrinology Unit, San Raffaele Hospital, Milano, Italy, <sup>7</sup>Medicine Diabetes Division, University of Texas Health Science Center, San Antonio, USA.

**Background and aims:** Autoantibodies against  $\beta$ -cell membrane proteins could directly mediate cytotoxicity in T1DM. Islet cell surface autoantibodies (ICSA) have been previously described but the nature of these autoantigens remained elusive. We have recently shown that the glial glutamate transporter (GLT1) is selectively expressed on the plasma membrane of human  $\beta$ -cells where it physiologically controls the extracellular glutamate concentration and prevents glutamate-induced  $\beta$ -cell death. We here explored the possibility that GLT1 could be a novel T1DM autoantigen and that putative anti-GLT1 autoantibodies could be pathogenic.

**Materials and methods:** A cell-based (GLT1-transfected cells) immunofluorescence, immunoprecipitation and Elisa assays were used to verify the presence of anti-GLT1 antibodies in serum samples from 43 T1DM patients and 35 healthy controls. We used indirect immunofluorescence, uptake and cell death assays to evaluate the possible immunopathological consequences of Ig binding to surface epitope of living target cells expressing GLT1. Data are expressed as mean value  $\pm$  SD. The study has been reviewed by the local ethics committee

**Results:** Using the cell-based immunofluorescence assay we found antibodies against GLT1 in 20 of 43 (46%) patients with T1DM and in 0 of 35 controls ( $p < 0.0001$ ; one-sided Fisher's exact test). Results were confirmed by ELISA assay performed on GLT1-expressing COS7 cells ( $p < 0.001$ ). T1DM but not control sera, immunoprecipitated a protein extracted from brain lysate, human islets of Langerans and GLT1-transfected COS7 cells of the same molecular weight of GLT1 and recognized by anti-GLT1 antibodies. Cluster and principal component analysis revealed that GLT1-immunoreactivity identified a novel subgroup of T1DM subjects, distinct from those identified by GADA, IA-2A and IAA. Exposure of  $\beta$ TC3 cells to GLT1-positive sera supplemented with active complement disrupted membrane integrity, increased Propidium iodide uptake ( $3.7 \pm 0.7$  fold increase; mean percentage of  $\beta$ -cells damaged by complement was  $17 \pm 5\%$  for GLT1-positive sera and  $3.6 \pm 0.2$  for control sera,  $p < 0.05$ ) and induced cell death. In the absence of complement, 9 out of 20 GLT1-positive T1DM sera (38%) severely downregulated the [<sup>3</sup>H]-D-Aspartic acid uptake in  $\beta$ TC3 cell cultures ( $> 60\%$  inhibition of uptake relative to untreated cells,  $p < 0.01$ ). Downregulation of GLT1 function was associated to protein internalization into endocytic/degradative pathway and led to increased apoptosis ( $4.6 \pm 0.3$  fold increase; mean percentage of TUNEL positive cells was  $15.3 \pm 3.5\%$  for 2 GLT1-positive sera and  $3.2 \pm 0.8$  for control sera).

**Conclusion:** GLT1 is a novel T1DM autoantigen and anti-GLT1 autoantibodies by binding to  $\beta$ -cells may initiate potentially pathogenic mechanisms including complement activation with  $\beta$ -cell membrane damage, as well as GLT1 down-regulation and disruption of glutamate homeostasis.

*Supported by: PUR2009*

## 479

### Clinical, metabolic and immunologic characteristics in adult patients with type 1 diabetes. Onset and short term prognosis over more than a decade in a Mediterranean area

A.M. de Hollanda<sup>1</sup>, C.M. Quirós<sup>1</sup>, A.J. Amor<sup>1</sup>, G.B. Aranda<sup>1</sup>, M. Vidal<sup>1</sup>, M. Jansà<sup>1</sup>, M. Giménez<sup>1</sup>, R. Casamitjana<sup>2</sup>, I.J. Conget<sup>1</sup>, E. Esmatjes<sup>1</sup>; <sup>1</sup>Diabetes Unit. Endocrinology Department, <sup>2</sup>Hormonology Unit, Hospital Clínic i Universitari, IDIBAPS, Barcelona, Spain.

**Introduction:** Around one third of new cases of type 1 diabetes (T1D) in Catalonia are diagnosed in subjects aged above 18 years. The aim of our study was to characterize a group of non-pediatric subjects with newly diagnosed T1D and to evaluate the short term prognosis of the disease under a specific treatment program and conventional intensive insulin therapy over more than a decade.

**Materials and methods:** We conducted a longitudinal, prospective, unicenter study including all subjects with an age  $\geq 18$  and  $\leq 40$  years recently diagnosed with T1D (1995-2010). Clinical characteristics, metabolic parameters, pancreatic  $\beta$  cell function (basal and glucagon stimulated c-peptide), and the presence of pancreatic autoantibodies (GAD, IA2 and IAA) were evaluated at onset and after 12 months of follow-up. All the subjects received our specific therapeutic education programme for patients with newly-diagnosed T1D.

**Results:** We included 271 patients at diagnosis, 62% women, mean age  $27 \pm 6.6$  years, 95% spanish native, with a duration of symptoms of  $8.9 \pm 9.7$  weeks. The diagnosis of T1D was performed in 28% of subjects in hyperglycemia, 52% in ketosis and 20% in ketoacidosis (KA). The mean BMI was of  $22.1 \pm 3.5$  kg/m<sup>2</sup>, HbA<sub>1c</sub>  $11.2 \pm 2.7\%$ , basal C-peptide  $0.7 \pm 0.4$  ng/mL, stimulated C-peptide  $1.3 \pm 0.7$  ng/mL. With respect pancreatic autoantibodies, GAD were positive in 77%, IA2 in 55% and IAA in 30% of subjects. Nine percent of the patients were negative for all three autoantibodies. KA at onset was associated with greater weight loss, higher HbA<sub>1c</sub> and lower C-peptide levels. After 12-months of follow-up, the BMI was  $23.9 \pm 3.7$  kg/m<sup>2</sup>, with a weight gain of  $4.6 \pm 8.6$  kg. On average, the HbA<sub>1c</sub> was  $6.8 \pm 3.6\%$ , with 67% of patients being  $< 7\%$ .  $\beta$  cell function remained unaltered in comparison with that observed at the onset of the disease ( $0.7 \pm 0.5$  and  $1.2 \pm 0.9$  ng/mL, basal and stimulated C-peptide, respectively). Regarding changes in clinical and metabolic characteristics at diagnosis and during the follow-up over the period of study, the only observed difference was a significant increase in HbA<sub>1c</sub> at onset ( $10.5 \pm 2.5\%$  to  $12.4 \pm 2.9\%$ ) from 1995-2000 to 2006-2010;  $p < 0.001$ ).

**Conclusion:** Our data collected from young adults with newly diagnosed T1D from a Mediterranean area indicates that the clinical presentation, metabolic and immunological characteristics, as well as, short-term prognosis has not substantially varied during more than a decade.

## 480

### Serum GADA detected by AID in type 2 diabetes reversely correlates to islet function

X. Wang<sup>1</sup>, M. Yin<sup>1</sup>, J. Yu<sup>1</sup>, J. Jia<sup>1</sup>, C.S. Hampe<sup>2</sup>;

<sup>1</sup>Department of Endocrinology, Jiangsu Province Hospital of TCM, Nanjing, China, <sup>2</sup>Department of Medicine, Department of Endocrinology, Jiangsu Province Hospital of TCM, Seattle, USA.

**Background and aims:** We hypothesize glutamate decarboxylase autoantibody (GADA) is induced in the background of type 2 diabetes (T2D) when islet  $\beta$  cells are damaged to shedding auto-antigens. The longer diabetic course and more severely impaired islet function will increase GADA titer. GADA in T2D patients is masked by its specific AID and not detected by routine assays. Our previously work demonstrated GADA in T2D patients could be recognized by its specific anti-idiotypic antibody (AID) in ELISA. We designed this study to investigate relationship between GADA and diabetic course and islet function in T2D.

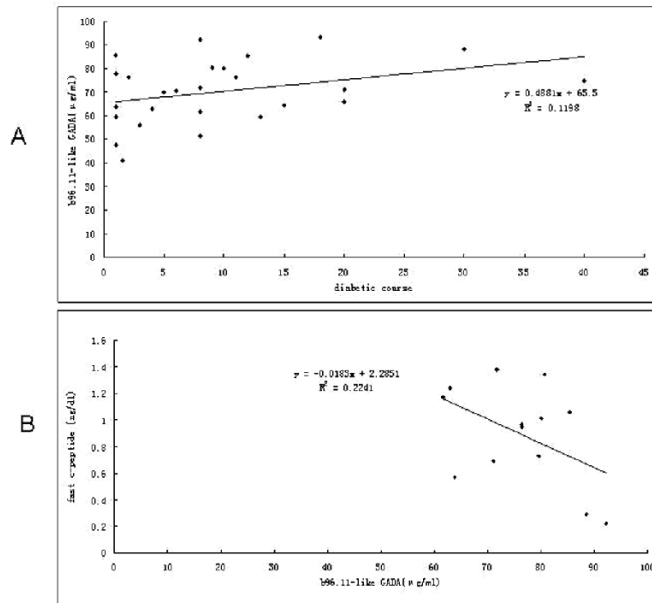
**Materials and methods:** Sera from 26 T2D were tested for GADA by routine luciferase immunoprecipitated assays (LIPS) and ELISA on immobilized human GADA specific AID. Sera immunoglobulins captured by AID covalently linked to protein A sepharose were analyzed by LIPS after elution. Monoclonal



human GADA b96.11 was used as standard or positive controls in all above assays. Relationships between GADA titer detected by ELISA with either diabetic course or fast C-peptide in 13 patients were evaluated by linear regression.

**Results:** GADA in all the samples is negative in routine LIPS but was detected in AID based ELISA. B96.11-like GADA concentration was  $70.32 \pm 13.49$  µg/ml in the 26 samples. Immunoglobulin bound to AID was proved to be GADA in LIPS after elution. Diabetic course is positively linear related with GADA tested by ELISA while fast C-peptide negatively linear correlates with GADA recognized by AID (Fig A and B).

**Conclusion:** Our findings suggest GADA exists in T2D and may be a marker of islet β cell function.



A: diabetic course positively related with GADA in T2D

B: fast C-peptide reversely related with GADA in T2D

Supported by: ADA 1-09-RA-64, NSF-C 81100555/H0707, grant 2006BAI04A03 of MST of China

## 481

**Natural Killer T cell IL-2 cytokine release is inhibited by the monoclonal autoantibody IC2 and might be restored by its anti-idiotypic antibodies**

K.L. Jensen<sup>1</sup>, I.D. Pedersen<sup>1</sup>, L. Åkesson<sup>2</sup>, Å. Lernmark<sup>2</sup>, I. Klöting<sup>3</sup>, F. Niessner<sup>3</sup>, J. Svalgaard<sup>1</sup>, K. Engkilde<sup>1</sup>, K. Buschard<sup>1</sup>, C.H. Brogren<sup>1</sup>;

<sup>1</sup>The Bartholin Institute, Rigshospitalet, Copenhagen, Denmark, <sup>2</sup>University Hospital Malmö, Sweden, <sup>3</sup>University Greifswald, Karlsburg, Germany.

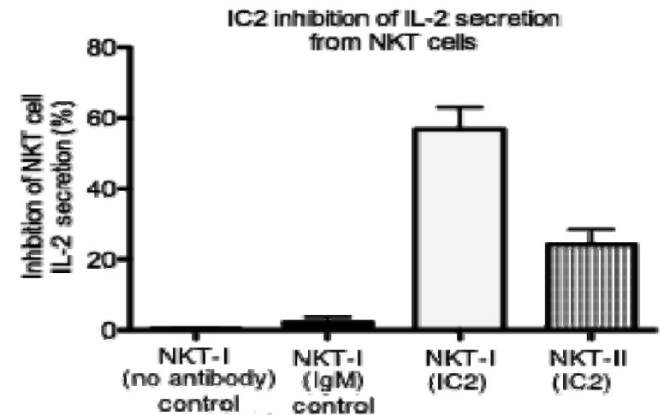
**Background and aims:** Identifying factors, which can prevent beta cell death is a major objective in diabetes research. The beta cell destruction in type 1 diabetes (T1D) is mediated by autoimmune T-cells under regulatory control of Natural Killer T cells (NKT cells) receiving stimulatory signals from certain CD1d-lipid complexes. It is thought that an inhibition of the stimulatory effects of NKT cells might be the reason for the degradation of beta cells in T1D. The aim of this study was to confirm the inhibitory role of the beta-cell surface specific monoclonal autoantibody IC2 on the regulatory NKT cells. This would mean that anti-idiotypic antibodies binding the paratope of IC2 could block the inhibitory effect of IC2 and thereby maintain the normal effect of NKT cells.

**Materials and methods:** We used a modified functional NKT-hybridoma assay with CD1d lipid complexes. One microgram of soluble mouse CD1d was coated on a flat-bottom 96-well plate for 18 hrs in 4 degrees in PBS. After washing 4 µg/ml IC2 + alfa-galactosyl-ceramide was mixed and added to the CD1d-coated plate and left for incubation at 37 degrees celsius for 4 hrs before addition of NKT 1.2 cells. For the NKT 19.3 cells 250µg/ml IC2 + lysosulfatide was mixed and added to the CD1d coated plate and left for incubation at 37 degrees celsius for 4 hrs before addition of NKT 19.3 cells. For both cell types, supernatants were collected after 17 hrs to measure IL-2 secretion by sandwich-ELISA.

**Results:** We show for the first time that IC2 can inhibit the IL-2 secretion from NKT type I and probably also from NKT type II cells. We found, the

inhibition most pronounced when IC2 was preincubated with alfa-galactosyl-ceramide before it was added to the CD1d coated plate, and the NKT cells then added subsequently. We saw that the addition of IC2 inhibited the IL-2 cytokine release of NKT type I cells with 56.8 %, when 4 µg/ml IC2 was added and NKT type II cells with 24.3 % when 250 µg/ml IC2 was added. When adding 4 µg/ml of rat IgM control, there was practically no inhibition, as expected (Figure).

**Conclusion:** According to our preliminary findings, the CD1d stimulation of type I NKT cells and to some degree also type II NKT cells can be inhibited by IC2. This makes the antibody interesting for future development of drugs preventing the development of autoimmune T-cell mediated diseases caused by a disturbance of the regulatory NKT cell signal. Furthermore, this suggests that the IC2 corresponding anti-idiotypic antibodies could have a preventive effect on T1D by blocking the IC2 inhibition of the NKT stimulation and thereby restoring the normal NKT cell activity.



Supported by: JDRF International and The EU-Fasilis Programme and ImmunoSigns

## PS 026 Beta cell channels

482

### Ca<sub>v</sub>1.3 L-type channel mediated Ca<sup>2+</sup> flux regulates human insulin release and feeds back to own mRNA expression

T.M. Reinbothe<sup>1</sup>, S. Alkayyal<sup>2</sup>, E. Ahlqvist<sup>2</sup>, T. Tuomi<sup>3</sup>, B. Isomaa<sup>4</sup>, V. Lyssenko<sup>2</sup>, E. Renström<sup>1</sup>;

<sup>1</sup>Islet Pathophysiology, Lund University Diabetes Centre, Malmö, Sweden,

<sup>2</sup>Diabetes and Endocrinology, Lund University Diabetes Centre, Malmö,

Sweden, <sup>3</sup>Helsinki University Central Hospital, Finland, <sup>4</sup>Biomedicum Helsinki, Folkhälsan Research Center, Finland.

**Background and aims:** L-type calcium channels are essential for pancreatic beta-cell function and they have been well studied in rodents. Here, we set out to determine which L-type channel isoform is operative in human pancreatic beta-cells and how it may be regulated in physiology and disease.

**Material and methods:** Ca<sub>v</sub>1.3 expression was compared in rat islets, INS1 832/13 cells and human islets using quantitative PCR and microarrays respectively and we assessed Ca<sub>v</sub>1.3 dependent insulin release in human islets. Human beta-cells were enriched using FACS and NewPortGreen. Phenotype/genotype associations of three single nucleotide polymorphisms (SNPs) were investigated in 8987 non-diabetic and 3298 type 2 diabetic subjects from Finland and Sweden. We used RNAi techniques to study the effect of Ca<sub>v</sub>1.3 knockdown on insulin release in INS1 832/13 cells and on exocytosis in human beta-cells.

**Results:** We find Ca<sub>v</sub>1.3 expression being the major L-type isoform in rat islets, the INS1 832/13 cells line, human islets and in FACS enriched human beta-cells on mRNA level (1130±23 Ca<sub>v</sub>1.3 transcripts vs. 19±1 Ca<sub>v</sub>1.2 transcripts). Protein is also mainly localizing to beta-cells within human islets. Ca<sub>v</sub>1.3 mRNA is less abundant in islets from diabetic subjects compared to controls (754±93 in cases vs. 1235±64 Ca<sub>v</sub>1.3 transcripts in controls; p=0.01) and this reduction in expression coincides with decreased human islet insulin release *in vitro*. Ca<sub>v</sub>1.3 knockdown reduced glucose stimulated insulin secretion in INS1 832/13 cells (3.8±0.4 ng/mg protein×h in siCa<sub>v</sub>1.3 treated vs. 6.7±0.7 ng/mg protein×h in control cells; p=0.0026) and exocytosis in human beta-cells (41±4 fF in siCa<sub>v</sub>1.3 treated vs. 68±8 fF in control cells; p=0.009) respectively. Genotype/phenotype association analyses revealed that the C allele of the CACNA1D gene SNP rs312480 was associated with reduced mRNA expression of the channel *in vitro* (CC: 1.4±0.1 vs. CT: 2.2±0.3 ng/islet×h p=0.04; T/T n.a.) and decreased insulin secretion *in vivo* (IVGTT, β=-0.085; p=0.027), whereas rs312486/G and rs9841978/G both associated with type 2 diabetes (OR=1.16 and 1.17; p=0.00115 and 6.4×10<sup>-5</sup>). Furthermore, we find high glucose *in vitro* to L-type channel dependently stimulate Ca<sub>v</sub>1.3 expression in human islets 1.7-fold (+/- 0.2) with the L-type channel blocker isradipine completely abolishing this effect (0.9-fold +/- 0.1). This raised Ca<sub>v</sub>1.3 expression contributes to increased intracellular calcium levels (2.12±0.04 AUC<sub>i</sub> for islets cultured for 24h at 20mM vs. 1.66±0.04 when cultured at 5 mM glucose; p=7×10<sup>-4</sup>).

**Conclusion:** The L-type Ca<sup>2+</sup> channel Ca<sub>v</sub>1.3 is important for appropriate glucose induced insulin secretion and deregulated expression as well as common variants in CACNA1D might contribute to type 2 diabetes.

**Supported by:** EU-network Cavnet, Linnaeus grant and EXODIAB (Swedish Research Council)

483

### Apolipoprotein CIII hyperactivates beta cell Ca<sub>v</sub>1 channels by coactivating PKA and Src kinase

Y. Shi<sup>1</sup>, G. Yang<sup>1</sup>, J. Yu<sup>1</sup>, L. Yu<sup>1</sup>, R. Westenbroek<sup>2</sup>, W.A. Catterall<sup>2</sup>, L. Juntti-Berggren<sup>1</sup>, P.-O. Berggren<sup>1</sup>, S.-N. Yang<sup>1</sup>;

<sup>1</sup>Department of Molecular Medicine and Surgery, Karolinska Institutet,

Stockholm, Sweden, <sup>2</sup>Department of Pharmacology, University of Washington, Seattle, USA.

**Background and aims:** Apolipoprotein CIII (ApoCIII) not only serves as an inhibitor of triglyceride hydrolysis, but also participates in diabetes-related pathological events. It has been demonstrated that elevated ApoCIII acts as a diabetogenic serum factor to drive β cell destruction via hyperactivation of voltage-gated Ca<sup>2+</sup> (Ca<sub>v</sub>) channels in the pancreatic β cell. ApoCIII can also bind to distinct cell surface receptors including scavenger receptor class B type I, Toll-like receptor 2 and uncharacterized binding sites relaying corresponding signals to their downstream effectors, e.g., β1 integrin, pertussis

toxin-sensitive G proteins, NF-κB and protein kinases. However, the molecular mechanisms whereby ApoCIII hyperactivates β cell Ca<sub>v</sub> channels are not known. This study aimed at mechanistically dissecting ApoCIII-induced hyperactivation of β cell Ca<sub>v</sub> channels at molecular level.

**Materials and methods:** Mouse islet β cells/insulin-secreting RINm5F cells and patch-clamp techniques/pharmacological manipulation/immunoblot analysis were employed.

**Results:** ApoCIII incubation significantly increased channel number, elevated open probability, prolonged mean open time and shortened mean closed time of single Ca<sub>v</sub>1 channels as compared with incubation with vehicle solution. Whole-cell Ca<sup>2+</sup> current densities in the ApoCIII group were significantly higher than those in the control group. In striking contrast, whole-cell Ca<sup>2+</sup> currents were similar between control cells and cells incubated with ApoCIII in the presence of the Ca<sub>v</sub>1 channel blocker nimodipine. The effect of ApoCIII was not significantly influenced by PKC inhibition. However, it was marginally reduced by individual inhibition of PKA or Src kinase. Importantly, either combined inhibition of PKA, PKC and Src kinase or coinhibition of PKA and Src kinase significantly counteracted the effect of ApoCIII on β cell Ca<sub>v</sub> channels. In addition, ApoCIII treatment did not alter β cell Ca<sub>v</sub>1 channel expression at the protein level.

**Conclusion:** ApoCIII selectively hyperactivates β cell Ca<sub>v</sub>1 channels through coactivation of PKA and Src kinase. ApoCIII-induced hyperactivation of β cell Ca<sub>v</sub>1 channels is characterized by enriched density and increased activity of functional Ca<sub>v</sub>1 channels in the β cell plasma membrane. This novel signal-transduction pathway may serve as an innovative drug target for the prevention of Ca<sup>2+</sup>-dependent β cell death in association with diabetes.

**Supported by:** EFSD grant, SSF, VR

484

### Multi-electrode arrays reveal glucose-dependent action potentials and slow waves that differ by their calcium sensitivity in mouse islet cells

F. Lebreton<sup>1</sup>, A. Caro<sup>1</sup>, A. Quotb<sup>2</sup>, J. Gaitan<sup>1</sup>, J. Laloum<sup>1</sup>, Y. Bornat<sup>2</sup>,

S. Renaud<sup>2</sup>, J. Lang<sup>1</sup>, M. Raoux<sup>1</sup>;

<sup>1</sup>Université Bordeaux 1, UMR CNRS 5248 - CBMN, Pessac, <sup>2</sup>UMR CNRS

5218, Université Bordeaux 1, IPB, Talence, France.

**Background and aims:** The β-cells of the islets of Langerhans generate electrical signals upon stimulation by glucose or other nutrients which are modulated by hormones. The ensuing increase in [Ca<sup>2+</sup>]<sub>i</sub> regulates important aspects such as exocytosis of secretory granules and gene expression. To overcome some limits of intracellular recordings, we have recently developed and published a novel electrophysiological approach based on the extracellular recording of action potentials (AP) with multi-electrode arrays (MEAs). This allowed for the first time long-term, non-invasive and multicellular recordings on islets. In the present work using this device, we report the existence, in addition to action potentials, of slower electrical signals of larger amplitude in islets.

**Materials and methods:** Clonal INS 832/13 β-cells and partially dissociated mouse islets were cultured for 2-4 days on MEAs containing 60 extracellular microelectrodes (Multichannel Systems, MCS). Electrical signals were recorded with a MEA1060-Inv-BC-Standard amplifier (MCS), with a band-pass of 0.1-3000 Hz, and were analysed off-line with MC\_Rack software (MCS).

**Results:** Electrophysiological studies in islets are generally conducted in the presence of high levels of extracellular Ca<sup>2+</sup> (~2.5 mM). Under these conditions, islet cells recorded on MEAs generated characteristic glucose-induced APs (40-60 ms duration, 10-50 μV). Decreasing Ca<sup>2+</sup> to more physiological conditions such as 1.2 mM or even to 0.4 mM, we observed the existence of glucose-induced slow electrical waves (SWs) (duration 800-1500 ms) with large amplitudes (30-200 μV). APs were present in 1.2 mM of external Ca<sup>2+</sup>, and with smaller amplitude in 0.4 mM Ca<sup>2+</sup>. Note that they were observed only during the presence of SWs. Like the APs, the SWs were absent at 3 mM of glucose, evoked in a reversible manner by 11 and 15 mM of glucose, and inhibited by both the L-type Ca<sup>2+</sup>-channel blocker nifedipine (25 μM) and adrenalin (5 μM). The SWs persisted during the application of 15 mM of glucose for up to 2 h with a mean frequency of 0.55±0.03 (n=24). Long-term recordings also revealed very regular oscillations in the SWs frequency with alternations of short silent phases and longer active phases. Interestingly, the same experiments performed with 0.4 to 5 mM of external Ca<sup>2+</sup> showed that the clonal INS 832/13 β-cells generate glucose-induced APs but not SWs. To gain insight into the nature of the SWs we started to investigate the putative involvement of multicellular processes in primary cells. Our preliminary results indicate that the gap junction inhibitor heptanol-1 (3.5 mM) abolished the SWs.

**Conclusion:** Our current observations extend our previous reports on the versatility of MEAs in islet research by the demonstration of two types of electrical signals evoked by glucose. Their difference in sensitivity to calcium suggests differences in their generation.

*Supported by:* ANR Emergence HY-BIOPACS, Région d'Aquitaine et FEDER «BIODIA»

## 485

### Role of anoctamin 1 (TMEM16A) as a volume regulated anion channel in insulin-producing cells

W.J. Malaisse<sup>1</sup>, M. Virreira<sup>2</sup>, Y. Zhang<sup>1</sup>, R. Crutzen<sup>2</sup>, N. Bulur<sup>1</sup>, P. Lybaert<sup>1</sup>, P.E. Golstein<sup>2</sup>, A. Sener<sup>1</sup>, R. Beauwens<sup>2</sup>;

<sup>1</sup>Laboratory of Experimental Hormonology, <sup>2</sup>Laboratory of Cell and Molecular Physiology, Université Libre de Bruxelles, Belgium.

**Background and aims:** Stimulation of insulin release by D-glucose entails two major ionic events, i.e. the closing of ATP-sensitive potassium channels prevailing at concentrations of the hexose in the range up to the threshold value for its insulinotropic action and the gating of volume regulated anion channels occurring mainly at higher concentrations of D-glucose. The present study aimed at investigating the possible role of anoctamin 1 (Ano1 or TMEM16A), a member of the TMEM16 or anoctamin family, as a volume regulated chloride channel in insulin-producing cells.

**Materials and methods:** RNA was isolated from rat and human islets, as well as BRIN-BD11 cells, for the study by RT-PCR of anoctamin 1, anoctamin 6 (Ano6 or TMEM16F) and anoctamin 10 (Ano 10 or TMEM16K) gene expression. Immunohistochemistry of pancreatic sections was performed using a standard ABC-DAB technique. For the measurement of insulin secretion, groups of 8 rat islets each prepared by the collagenase method were incubated for 90 min in a salt-balanced medium containing bovine serum albumin. Voltage measurements in BRIN-BD11 cells were performed in nystatin-perforated whole cell configuration patch clamp experiments.

**Results:** Ano1 mRNA was found in rat and human islets, with a lower level in BRIN-BD11. In rat islets Ano1 and Ano6 were predominant, with a lower level of Ano10. Immunohistochemistry confirmed the presence of Ano1 in mouse, rat and human pancreatic islets. In rat isolated pancreatic islets incubated in the presence of 16.7 mM D-glucose, tannic acid (TA), inhibitor of Ano1, caused a concentration-related decrease ( $p < 0.01$ ) of insulin output ( $\mu\text{U}/\text{islet}$ ;  $n=22-24$ ) from a control value of  $303 \pm 16$  to  $245 \pm 13$  (10  $\mu\text{M}$  TA),  $227 \pm 14$  (30  $\mu\text{M}$  TA),  $175 \pm 11$  (50  $\mu\text{M}$  TA) and  $154 \pm 14$  (100  $\mu\text{M}$  TA), as compared to a basal value recorded in the sole presence of 2.8 mM D-glucose of  $43 \pm 4$ . Tannic acid (100  $\mu\text{M}$ ) also opposed the spiking activity and depolarization of the plasma membrane evoked by exogenous  $\text{H}_2\text{O}_2$  (100  $\mu\text{M}$ ) in BRIN-BD11 cells exposed to 5.0 mM D-glucose, the latter observation being consistent with the role recently attributed to NAD(P)H oxidase-derived  $\text{H}_2\text{O}_2$  as an activator of volume regulated anion channels in insulin-producing cells.

**Conclusion:** The present findings support the view that anoctamin 1 (TMEM16A) indeed acts as a volume regulated channel in insulin-producing cells.

## 486

### Influence of miR-375 on electrophysiological properties of voltage-dependent $\text{Na}^+$ channels in mouse beta cells and INS1-832/13 cells

V.A. Salunkhe<sup>1</sup>, M. Braun<sup>2</sup>, J.L.S. Esguerra<sup>1</sup>, I. Mollet<sup>1</sup>, A. Wendt<sup>1</sup>, M. Stoffel<sup>3</sup>, P. Rorsman<sup>4</sup>, L. Eliasson<sup>1</sup>;

<sup>1</sup>Lund University Diabetes Centre, Clinical Research Centre Malmö, Lund University, Malmö, Sweden, <sup>2</sup>Alberta Diabetes Institute, University of Alberta, Edmonton, Canada, <sup>3</sup>Institute of Molecular Systems Biology, Swiss Federal Institute of Technology, ETH Zurich, Switzerland, <sup>4</sup>Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, UK.

**Background and aims:** Glucagon secretion from alpha-cells is dependent on voltage-dependent  $\text{Na}^+$  ( $\text{Na}_v$ ) channels. Both pancreatic alpha- and beta-cells are equipped with these channels which are known to exhibit cell-type specific inactivation properties. MicroRNAs control protein expression and the islet-abundant miR-375 has target genes known to be involved in insulin secretion. We aim to investigate possible targets of miR-375 among  $\text{Na}_v$  channel subunits (gene names  $\text{SCN}$ x) and its subsequent effect on  $\text{Na}_v$  currents properties in beta-cells.

**Materials and methods:** Whole-cell configuration patch-clamp technique was used to investigate the inactivation properties of the  $\text{Na}_v$ -current in a) beta-cells from control and miR-375 knockout mice (375KO) and b) INS1-

832/13 cells (rat beta-cell line) transfected with FITC- labelled locked nucleic acid (LNA) against miR-375 (anti-miR-375) or scramble LNA oligonucleotide (control). Glucose stimulated insulin secretion assay and RT-qPCR was also performed using INS1-832/13 cells to investigate expression levels of  $\text{Na}_v$  channel subunits during glucose stimulation. TargetScan was used to predict miR-375- $\text{SCN}$ x interactions.

**Results:** The half-maximal inactivation ( $V_h$ ) of  $\text{Na}_v$ -current was  $-83 \pm 1$  mV ( $n=24$ ) in control mice beta-cells. This was shifted to more positive membrane potential in the 375KO ( $V_h = -69 \pm 2$  mV;  $n=30$ ;  $p < 0.001$ ). Thus, in the absence of miR-375 mice beta-cells  $\text{Na}_v$  channel properties becomes more alpha-cell like. In INS1-832/13 cells, the  $V_h$  of  $\text{Na}_v$ -current was  $-57 \pm 2$  mV ( $n=12$ ) in control cells. This was shifted to more negative membrane potential upon treatment with anti-miR-375 ( $V_h = -62 \pm 1$  mV;  $n=25$ ;  $p < 0.01$ ). The  $\text{Na}_v$  current is consistent with the flow of  $\text{Na}^+$  through different  $\text{Na}$ -channel subtypes and miR-375 might target one of these subtypes specifically present in beta-cells. Indeed, computational predictions identified a number of  $\text{Na}_v$  channel subunits as putative targets in species-specific manner, e.g.  $\text{SCN3A}$  in rat and human while  $\text{SCN3B}$  in mouse. Upon glucose stimulation of INS-1 832/13 cells, we observed inverse correlation of miR-375 and  $\text{SCN3A}$  expression levels suggesting possible regulatory interactions.

**Conclusion:** We found direct influence of miR-375 on the electrophysiological properties of  $\text{Na}_v$  channel in mouse beta-cells and INS-1 832/13 cells. The observed shift in half-maximal inactivation of  $\text{Na}_v$ -current in opposite directions was possibly due to species-specific regulation of putative  $\text{Na}_v$  channel subunit targets of miR-375. We conclude that miR-375 apart from being important in exocytosis also has potential roles in the modulation of beta-cell ion channels.

*Supported by:* Swedish Research Council

## 487

### Scn3a encodes the functionally important $\text{Na}^+$ -channel alpha-subunit (Nav 1.3) in mouse pancreatic alpha and beta cells

Q. Zhang<sup>1</sup>, R. Ramracheya<sup>1</sup>, M. Bengtsson<sup>1</sup>, M. Braun<sup>2</sup>, A. Welling<sup>3</sup>, P. Rorsman<sup>1</sup>;

<sup>1</sup>OCDEM, Oxford, UK, <sup>2</sup>Alberta Diabetes Institute, Edmonton, Canada,

<sup>3</sup>Institut für Pharmakologie und Toxikologie, München, Germany.

**Background and aims:** Insulin-secreting  $\beta$ - and glucagon-producing  $\alpha$ -cells are equipped with voltage-gated  $\text{Na}^+$ -channels ( $\text{Na}_v$  channels).  $\text{Na}_v$  channels are involved in the hormone secretion from pancreatic islet endocrine cells due to its role in the generation of action potentials. This study is to dissect different  $\text{Na}_v$  channel  $\alpha$ -subunits (pore forming subunits) expressed in  $\alpha$ - and  $\beta$ -cells and to understand their involvement in glucagon and insulin secretion.

**Material and methods:** The functional role of the different  $\text{Na}^+$ -channel  $\alpha$ -subunits was analyzed by electrophysiological recordings (patch-clamping) and hormone release measurements in wildtype,  $\text{Scn3a}/\text{Na}_v 1.3$  and  $\text{Scn9a}/\text{Na}_v 1.7$  knockout mice. Molecular identity of  $\text{Na}_v$  channel  $\alpha$ -subunits was confirmed by using single cell RT-PCR and quantitative RT-PCR in mouse and human islets, respectively.

**Results:** Both  $\alpha$ - and  $\beta$ -cells are in possession of rapid inactivating  $\text{Na}^+$  currents. The steady state of inactivation of  $\text{Na}^+$  currents exhibits a biphasic pattern in all  $\alpha$ -cells and 30% of  $\beta$ -cells. In all  $\alpha$ -cells ~70% of  $\text{Na}^+$  currents inactivate at positive potential ( $V_{0.5} = -47$  mV). In  $\beta$ -cells with biphasic inactivation, 50%  $\text{Na}^+$  currents was found to have similar inactivation at -50mV. 70% of  $\beta$ -cells tested exhibited a monophasic inactivation with the  $V_{0.5}$  of -97mV. Application of a broad spectrum  $\text{Na}_v$  channel blocker, tetrodotoxin (TTX), inhibited glucagon secretion when islets were sensing low glucose. The same blocker had no effect on glucose stimulated insulin secretion. At the molecular level, human and mouse pancreatic islets express similar  $\text{Na}^+$ -channel subunit complements (principally  $\text{SCN3A}/\text{Scn3a}$ ,  $\text{SCN8A}/\text{Scn8a}$ ,  $\text{SCN9A}/\text{Scn9a}$ ,  $\text{SCN1B}/\text{Scn1b}$  and  $\text{SCN3B}/\text{Scn3b}$ ). Ablation of  $\text{Scn3a}/\text{Na}_v 1.3$  resulted in the suppression of the  $\text{Na}^+$ -current active at physiological voltages in both  $\alpha$ - and  $\beta$ -cells and inhibited glucagon and insulin secretion triggered by low and high glucose, respectively. Cell-specific ablation of  $\text{Scn9a}^{-/-}/\text{Na}_v 1.7$  in  $\alpha$ - or  $\beta$ -cells did not affect glucagon or insulin secretion but led to the selective removal of a  $\text{Na}^+$ -current component inactivating at membrane potentials more negative than -70 mV in  $\beta$ -cells whereas only small effects were seen in  $\alpha$ -cells. These data suggest that Nav1.3 represents the functionally important  $\text{Na}^+$ -channel  $\alpha$ -subunit in  $\alpha$ - and  $\beta$ -cells.

*Supported by:* Wellcome trust



## 488

**Activation of nonselective cation channels via cAMP-EPAC2 pathway is involved in exendin-4 potentiated insulin secretion**M. Yoshida<sup>1</sup>, S. Yamato<sup>1</sup>, K. Dezaki<sup>2</sup>, M. Nakata<sup>3</sup>, M. Kawakami<sup>1</sup>, T. Yada<sup>3</sup>, M. Kakei<sup>1</sup><sup>1</sup>Internal Medicine <sup>2</sup>Physiology <sup>3</sup>Integrated Physiology, Jichi Medical University, Shimotsuke, Japan.

**Background and aims:** Glucagon-like peptide 1 (GLP-1) stimulates glucose-dependently insulin secretion that is brought about by increase in the intracellular  $\text{Ca}^{2+}$  concentration in a dose-dependent manner at supra-threshold concentration of glucose. There are several lines of evidence that GLP-1 influences ion channels as a mechanism of enhancement of insulin secretion, but all of these are not confirmatory yet. Here we report a novel mechanism of GLP-1 on background non-selective cation channel current (NSCC) in rat pancreatic beta cells.

**Materials and methods:** Perforated whole-cell patch-clamp technique was used in rat pancreatic beta cell. Insulin secretion from isolated islets was measured using ELISA-kit in static incubation.

**Results:** Insulin secretion in the presence of 11.2 mM glucose was increased by  $10^{-9}$  M exendin-4 from  $21.2 \pm 1.48$  ng/10 islets to  $30.9 \pm 1.85$  ng/10 islets (mean  $\pm$  sem,  $P=0.0011$ , unpaired test). The resting membrane conductance that was measured by ramp clamp from -100 mV to -50 mV at 2.8 mM glucose was  $686 \pm 320$  pS/pF ( $n=5$ ), of which the majority of currents were due to ATP-sensitive  $\text{K}^{+}$  channels and this was decreased to  $31.5 \pm 7.6$  pS/pF ( $n=7$ ) by changing to 5.6 mM glucose with 100  $\mu\text{M}$  tolbutamide ( $P<0.05$ , unpaired test). The resting conductance was further increased to  $62.2 \pm 15.7$  pS/pF ( $n=7$ ,  $P<0.05$  with paired test) after addition of  $10^{-10}$  M exendin-4 that is a GLP-1 receptor agonist. Exendin-4 greater than  $10^{-11}$  M elicited inward current at the holding potential of -70 mV and exendin-4 sensitive current reversed around 0 mV, indicating that exendin-4 activated NSCC. The increase in NSCC by exendin-4 was dose-dependent at the concentrations between  $10^{-11}$  M and  $10^{-9}$  M and conversely decreased these increasing effects on NSCC at concentrations greater than  $10^{-9}$  M. The NSCC sensitive to exendin-4 was inhibited by 10  $\mu\text{M}$  ruthenium red, nonspecific blocker of transient receptor potential channels. In the presence of  $10^{-7}$  M exendin-9-39, GLP-1 receptor antagonist, exendin-4 at  $10^{-10}$  M was without effect on NSCC. This indicates involvement of GLP-1 receptor binding followed by activation of NSCC. Dibutyl-*c*-AMP at 1 mM activated the NSCC. In the presence of 10  $\mu\text{M}$  H89, protein-kinase A inhibitor,  $10^{-10}$  M exendin-4 increased NSCC. 8-pCPT-2-*O*-Me-*c*-AMP at 10  $\mu\text{M}$ , EPAC2 activator, also increased the NSCC by an extent similar to that on exposure to exendin-4. We observed that liraglutide, GLP-1 analogue, increased the inward current at -70 mV at  $10^{-10}$  M.

**Conclusion:** Activation of NSCC, presumably a class of transient receptor channel, via cAMP-EPAC2 pathway is involved in GLP-1-mediated beta cell stimulation.

## 489

**Pharmacology of GABAA channels in human pancreatic islets**

Y. Jin, S. Korol, Z. Jin, B. Birnir; Uppsala University, Sweden.

**Background and aims:** Gamma-aminobutyric acid (GABA) is known as the inhibitory neurotransmitter in the adult mammalian central nervous system (CNS). It has also been shown to be secreted from the islets of Langerhans in pancreas where it serves as an important paracrine and autocrine signaling molecule regulating the pancreatic cells excitability through GABAA channels. The GABAA channel is a ligand-gated ion channel permeable to chloride and bicarbonate. When the GABAA channels are activated, they depolarize the  $\beta$ -cell and hyperpolarize the  $\alpha$  and  $\delta$ -cell in the human islets, due to different intracellular chloride concentrations. In pancreatic islets, GABA is synthesized in the  $\beta$ -cells, and released by both vesicular and nonvesicular processes. We have recently shown GABA effectively modulates hormone release in islets from type 2 diabetic and normoglycaemic individuals. Moreover, both phasic and tonic GABAA channels activation were recorded in intact islets. However, the interstitial GABA concentration is not known, and information regarding the islets GABAA channel pharmacology is not available.

**Materials and methods:** We, therefore, applied whole-cell patch-clamp recordings on intact human islets to further investigate the pharmacological characteristics of the native GABAA channels expressed in the islets.

**Results:** The results show that the GABAA channels opening in human pancreatic islets are enhanced relative to the baseline activity when 100 nM GABA is applied but the channels do desensitize in 1  $\mu\text{M}$  GABA. We further examined several known GABAA channel modulators: pentobarbital, diazepam, zolpidem and propofol. The results demonstrated that pentobarbital, diazepam and propofol consistently potentiated the GABAA channels, whereas zolpidem did not, which differs from the pharmacology in CNS.

**Conclusion:** These results support the notion that GABAA channels with very high affinity are present in the islets. Furthermore, the endogenous intra-islet GABA concentration is lower than 100 nM in islets when 20 mM glucose is present. Considering our gene expression data, interestingly, in human islets the  $\pi$  subunit has a high expression level whereas in CNS it is very low. From human genome analysis, the  $\pi$  subunit has a high similarity to the  $\delta$  subunit, a classical extrasynaptic subunit. The incorporation of the  $\pi$  may possibly be the reason for different pharmacological pattern observed in the islets as compared to the CNS, which might potentially be valuable to serve as a novel drug target

Supported by: exodiab

## 490

**Insulin secretion induced by potassium depolarisation - just a matter of Nernst equilibrium?**

M. Willenborg, M. Belz, K. Schumacher, I. Rustenbeck;

Institute of Pharmacology, Toxicology and Clinical Pharmacy, Technical University of Braunschweig, Germany.

**Background and aims:** The elevation of the external potassium concentration is a widely used maneuver to elicit insulin secretion without affecting energy metabolism or  $\text{K}_{\text{ATP}}$  channel function. The resulting depolarization is thought to be the inevitable consequence of the shift of the Nernst equilibrium. Recently we observed that 15 mM KCl was ineffective to induce insulin secretion despite clearly depolarizing the plasma membrane and increasing  $[\text{Ca}^{2+}]_i$ . By contrast 40 mM, which is commonly used in the field, had a drastic insulinotropic effect accompanied by a stronger depolarization and further elevation of the  $[\text{Ca}^{2+}]_i$ . So the question arose if these different insulinotropic effects of low and high external potassium are just of quantitative or rather qualitative nature.

**Materials and methods:** Membrane potentials and currents of separated NMRI mouse  $\beta$ -cells were measured in the perforated patch mode of patch-clamp technique, cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) of NMRI mouse islets was measured by the Fura method. Insulin secretion was measured by batch perfusion of pancreatic islets. All parameters except electrophysiological experiments were performed at 37°C. Patch-clamp experiments were done at room temperature.

**Results:** In presence of a basal glucose concentration 15 mM KCl depolarized pancreatic beta cells by 20.7 mV and led to an increase of  $[\text{Ca}^{2+}]_i$  which was not inferior to an increase induced by 20 mM glucose. However these changes did not result in an increase of insulin secretion. By contrast 40 mM KCl induced a 6-fold increase of secretion. Under these conditions beta cells were depolarized by 41.2 mV accompanied by a stronger increase of  $[\text{Ca}^{2+}]_i$  than performed by 15 mM KCl. To rule out a subthreshold increase of  $[\text{Ca}^{2+}]_i$  by 15 mM KCl, 10  $\mu\text{M}$  cyclopiazonic acid was applied prior to KCl application resulting in an increase of  $[\text{Ca}^{2+}]_i$  comparable to the plateau performed by 40 mM KCl but without stimulatory effect on secretion. Next the effect of the selective L-type calcium channel blocker Nifedipine and the nonselective blocker  $\text{Co}^{2+}$  on membrane potential, -current and  $[\text{Ca}^{2+}]_i$  was tested. 10  $\mu\text{M}$  Nifedipine reduced the KCl induced depolarization by 1.2 mV in case of 15 mM  $\text{K}^{+}$  and by 1.5 mV in case of 40 mM  $\text{K}^{+}$ . 2.5 mM  $\text{Co}^{2+}$  caused a repolarization of 2.9 and 6.1 mV respectively, indicating that high KCl might increase  $[\text{Ca}^{2+}]_i$  not only via activated L-type VDCC. In concordance the effect of Nifedipine on  $[\text{Ca}^{2+}]_i$  was more marked at low  $\text{K}^{+}$  than at high  $\text{K}^{+}$  induced depolarization. Finally the membrane potential was clamped at -30 mV, which is typically reached by 40 mM  $\text{K}^{+}$ . In presence of Tolbutamide and TEA spontaneous electrical activity in form of inward action currents with peak values of 30 pA was observed, which could be abolished by Nifedipine. Subsequent application of 40 mM  $\text{K}^{+}$  transformed this inward spiking into a continuous inward current of  $\approx 20$  pA which was marginally sensitive to Nifedipine application but could be clearly inhibited by  $\text{Co}^{2+}$ .

**Conclusions:** In the present study we could show that the seemingly simple tool potassium induces insulin secretion not only by shifting the Nernst equilibrium but also modulating membrane conductance. It transforms regular inward action currents into continuous Nifedipine insensitive ion fluxes. This might be the reason why strong potassium induced depolarizations do not show action potential spiking while drastically increasing  $[\text{Ca}^{2+}]_i$ .

Supported by: DDG, DFG, DDS

## PS 027 Cytokines and beta cell damage

491

### Activation of the cholinergic anti-inflammatory pathway to protect pancreatic beta cells against cytotoxic attacks

P. Klee, E. Somme, A. Guerardel, V.M. Schwitzgebel;

Department of Pediatrics, University Hospital of Geneva, Switzerland.

**Background and aims:** Type 1 diabetes (T1DM) results from an auto-immune destruction of more than 90% pancreatic  $\beta$ -cells, leaving few survivors that are insufficient to properly regulate blood glucose. We consider the possibility that the activation of nicotinic acetylcholine (nACh) receptors may protect  $\beta$ -cells from auto-immunity. nACh receptors are channels formed by 5 subunits that form a pathway for ions across the cell membrane. They have been implicated in the control of inflammation, through activation of their  $\alpha 7$  subunit and allow the central nervous system, by activation of efferent nerve fibers, to modulate peripheral inflammation - a mechanism termed "cholinergic anti-inflammatory pathway". nACh receptors are expressed in pancreatic  $\beta$ -cells, but no study has analyzed the effect of their activation on the resistance of  $\beta$ -cells against toxins relevant in the pathogenesis of T1DM. The aim of this work was to test whether the activation of nACh receptors could be implicated in the protection of  $\beta$ -cells exposed to toxins mimicking the environment of a  $\beta$ -cell at the onset of T1DM.

**Materials and methods:** Islets isolated from wild type (WT) mice, as well as from mice knocked out for the  $\alpha 7$  or  $\beta 2$  subunit of nACh receptors were cultured in RPMI medium with or without nicotine or Choline. After 24h, they were exposed during another 24h to a cocktail of IL-1 $\beta$  + TNF $\alpha$  + IFN $\gamma$ , a cocktail of cytokines often used to mimic the surrounding of a  $\beta$ -cell at the onset of T1DM. Cell death was then measured by TUNEL analysis of islet sections. Nitrites, a stable degradation product of nitric oxide (NO) were measured in the culture medium by a Griess reaction.

**Results:** The exposure of WT islets to the above-mentioned cocktail of cytokines showed increased cell death, as compared to untreated islets. Pre-culture with nicotine during 24h significantly decreased the effect of cytokines. Exposure of islets isolated from mice knocked out for either the  $\beta 2$  or the  $\alpha 7$  subunit of nACh receptors to the same cytokines showed that nicotine significantly decreased cytokine-induced  $\beta$ -cell apoptosis also in absence of the  $\beta 2$  subunit. In contrast, this protection was lost in islets isolated from mice knocked out for the  $\alpha 7$  subunit, indicating that the  $\alpha 7$  subunit is necessary for the action of nicotine. To confirm the importance of this subunit, we pre-incubated islets isolated from WT mice to choline, a specific agonist of this subunit. We found that this substance significantly decreased cytokine-induced  $\beta$ -cell death. Finally, measurement of nitrite accumulation in the culture media showed that pre-incubation with nicotine did not alter the production of NO by  $\beta$ -cells, indicating that cholinergic agonists did not interfere with the signaling pathways underlying cytokine receptors.

**Conclusion:** Our results show that activation of the  $\alpha 7$  subunit of nACh receptors, a subunit thought to be part of the cholinergic anti-inflammatory pathway and previously implicated in the regulation of cell death, decreased  $\beta$ -cell apoptosis induced by a cocktail of cytokines thought to mimic the environment of a  $\beta$ -cell at the onset of T1DM. This effect was not linked to alteration of NO production by  $\beta$ -cells, an event occurring early in the signaling pathway connecting cytokine signaling and apoptosis. This opens the interesting perspective of a pharmacological modulation of  $\beta$ -cell death to prevent the massive destruction leading to T1DM.

Supported by: the Wolfermann-Nägeli Foundation, Zürich, CH

492

### Interleukin-6 potentiates the cytotoxic actions of both interleukin-1 $\beta$ and palmitate in pancreatic beta cells

M.A. Russell, N.G. Morgan;

Institute of Biomedical and Clinical Science, Peninsula College of Medicine and Dentistry (University of Exeter), Plymouth, UK.

**Background and aims:** Interleukin (IL)-6 and IL-1 $\beta$  are elevated within the islet milieu in human type 2 diabetes and may contribute to the low grade inflammation associated with this condition. IL-6 has well-recognised actions on pancreatic  $\alpha$ -cells and it has also been implicated in the development of insulin resistance at extra-pancreatic sites. However, the effect of this cytokine

on the viability of  $\beta$ -cells has received relatively little attention. Therefore, in the current work we have studied this.

**Materials and methods:** INS-1E cells were used as a model system but some experiments were also repeated with isolated human islets. Cell viability was assessed by flow cytometry following propidium iodide staining, whilst nitrite levels were determined by Griess assay. Data are presented as mean values ( $\pm$  SEM).

**Results:** The IL-6 receptor comprises two protein subunits (IL-6Ra and GP130) and the expression of these molecules was studied at the mRNA level by RT-PCR, using RNA prepared from INS-1E cells and human islets. Both subunits were expressed. Functionality of the receptor was confirmed by treatment of cells with IL-6. This resulted in enhanced tyrosine phosphorylation of the transcription factor, STAT3 within 15 minutes. Changes in phosphorylation of a second STAT protein, STAT6, are not typically associated with IL-6 mediated responses but, surprisingly, phosphorylation of STAT6 was also increased upon exposure to IL-6 in INS-1E cells. Exposure of INS-1E cells to IL-6 alone did not influence their viability, however the cytokine potentiated the cytotoxic effects of IL-1 $\beta$  (IL-1 $\beta$ :  $14.3 \pm 0.2$  % dead cells, IL-1 $\beta$  + IL-6:  $22.2 \pm 0.7$  %,  $p < 0.001$ ). Similarly, the response to a pro-inflammatory cytokine mix (pro:  $16.3 \pm 0.3$  % dead cells, pro + IL-6:  $22.7 \pm 0.7$  %,  $p < 0.001$ ) the saturated fatty acid, palmitate (palm:  $37.4 \pm 0.8$  % dead cells; palm + IL-6:  $44.3 \pm 0.3$  %,  $p < 0.001$ ) and serum withdrawal (serum free:  $45.5 \pm 1.1$  % dead cells, IL-6:  $60.7 \pm 1.3$  %,  $p < 0.001$ ) was also increased by IL-6. Nitrite production (an index of nitric oxide formation) was not elevated when cells were treated with IL-6 alone, whereas IL-1 $\beta$  caused a rise in nitrite formation and this response was potentiated by IL-6 (IL-1 $\beta$ :  $17.5 \pm 0.3$   $\mu$ M nitrite, IL-1 $\beta$  + IL-6:  $21.8 \pm 0.4$   $\mu$ M,  $p < 0.001$ ). We, and others, have shown that the anti-inflammatory cytokines IL-4 and IL-13 can exert cytoprotective effects in  $\beta$ -cells but it is not known whether they influence viability in the presence of IL-6. We found that both IL-4 and IL-13 improved the viability of cells treated with IL-6 and IL-1 $\beta$  (IL-1 $\beta$  + IL-6:  $35.3 \pm 0.9$  % dead cells, IL-1 $\beta$  + IL-6 + IL-4:  $29.4 \pm 0.9$  %,  $p < 0.001$ , IL-1 $\beta$  + IL-6 + IL-13:  $29.4 \pm 1.4$  %,  $p = 0.002$ ). Nitrite production under these conditions was also attenuated (IL-1 $\beta$  + IL-6 + IL-4:  $85.1 \pm 1.9$  % of nitrite produced following IL-6 + IL-1 $\beta$  treatment,  $p = 0.005$ ; IL-1 $\beta$  + IL-6 + IL-13:  $77.3 \pm 2.5$  %,  $p < 0.001$ ).

**Conclusion:** IL-6 augments the cytotoxic effects of pro-inflammatory cytokines, serum withdrawal and the saturated fatty acid palmitate in pancreatic  $\beta$ -cells. Since pancreatic islet cells may be exposed to elevated levels of IL-1 $\beta$ , IL-6 and fatty acids in type 2 diabetes, these may act in combination to promote a reduction in  $\beta$ -cell mass thereby exacerbating the condition. Anti-inflammatory cytokines (IL-4 and IL-13) antagonised the loss of viability in cells exposed to IL-6, suggesting that the precise cytokine milieu will be important in determining the ultimate fate of the  $\beta$ -cells *in vivo*.

493

### Activities and modulation of the proteasome in human pancreatic islets

M. Bugliani, F. Syed, M. Suleiman, F. Filippini, U. Boggi, L. Marselli, P. Marchetti;

University of Pisa, Italy.

**Background and aims:** The proteasome (Prot) is responsible of the proteolytic activity of the ubiquitin-proteasome system, that degrades unneeded or damaged proteins. Sparse data in clonal cell lines and rodent models suggest that Prot alterations may be associated with changes in beta cell function and survival. In this study we assessed the activities of Prot in human pancreatic islets and their regulation by several agents and conditions, including the presence of type 2 diabetes (T2D).

**Materials and methods:** Islets were isolated from 16 non-diabetic (ND; age  $67 \pm 16$  yrs, males/females 8/8, BMI  $25.2 \pm 1.6$  kg/m<sup>2</sup>) and 12 T2D (age  $74 \pm 6$  yrs, males/females 10/2, BMI  $27.5 \pm 2.39$  kg/m<sup>2</sup>) subjects. Proteolytic (trypsin-like, chymotrypsin-like and caspase-like) activities were measured by luminometric methods in whole islets or isolated (by affinity matrix beads) proteasome and the effects were evaluated of 0.5 mM palmitate (24h incubation), cytokines (IL-1 $\beta$ , 50 U/ml and IFN  $\gamma$ , 1000 U/ml, 24h incubation), presence of T2D and exposure to 2.4  $\mu$ g/ml metformin or 10 ng/ml GLP-1. Glucose-stimulated insulin secretion was assessed by batch incubation.

**Results:** All the proteasome activities were found in human islets and confirmed with isolated proteasome. In ND islets, chymotrypsin- and caspase-like activities were 2-fold (or more) higher than trypsin-like activity. Palmitate exposure decreased proteasome activities by 30-40% ( $p < 0.05$ ) and cytokines induced a 40% increase of trypsin-like activity ( $p < 0.01$ ). In T2D islets all the activities (in particular the trypsin-like) were reduced of 30-50% ( $p < 0.05$  or

less) compared to ND islets. Incubation (24h) of T2D islets with metformin or, more markedly so, GLP-1 determined a significant ( $p<0.05$ ) 20–40% increase of trypsin-like activity, which was associated with improved ( $p<0.05$ ) glucose-stimulated insulin secretion (stimulation index from  $1.5\pm0.2$  in untreated T2D islets to  $1.8\pm0.3$  after metformin and  $1.9\pm0.3$  after GLP-1).

**Conclusion:** These results show the presence of all the Prot activities in human islets, that are differently modulated by stressors such as palmitate or cytokines; Prot activities are reduced in T2D islets, and can be increased by some antidiabetic drugs (metformin and GLP-1), an effect that is associated with improved insulin secretion. Therefore the proteasome may play an important role in the pathophysiology of the beta cell and in its protection by pharmacological intervention.

## 494

### Transcription and activity of the proteasome are induced by pro-inflammatory cytokines, and proteasome expression is inhibited by HDAC and deubiquitinase inhibition

M. Lundh<sup>1,2</sup>, A. Choudhary<sup>2,3</sup>, D.H.C. Chou<sup>4</sup>, A. Tang<sup>2</sup>, T. Mandrup-Poulsen<sup>1,5</sup>, B. Wagner<sup>2</sup>;

<sup>1</sup>Department of Biomedical Sciences, University of Copenhagen, Denmark,

<sup>2</sup>Broad Institute of MIT and Harvard, Cambridge, USA, <sup>3</sup>Society of Fellows, Harvard University, Cambridge, USA, <sup>4</sup>Massachusetts Institute of Technology, Cambridge, USA, <sup>5</sup>Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** Selective destruction of pancreatic beta-cells causes Type 1 diabetes mellitus (T1DM). Pro-inflammatory cytokines e.g. IL-1 $\beta$ , TNF $\alpha$  and IFN $\gamma$  are secreted by infiltrating immune cells and induce beta-cell apoptosis. Cytokine-induced beta-cell apoptosis is prevented by inhibition of lysine deacetylases (KDACs) or by the inhibition of deubiquitinases (DUBs). Several lines of evidence indicate the importance of the proteasome in cytokine-induced beta-cell death, but the regulation of the expression and the mechanism of activation of the proteasome are unknown. Furthermore, it is unknown whether the protective effects of KDAC and DUB inhibition are mediated via effects on the proteasome.

**Materials and methods:** INS-1E cells were exposed to a combination of pro-inflammatory cytokines (IL-1 $\beta$ , TNF $\alpha$  and IFN $\gamma$ ) for 3, 6, 9, 12 or 24 hours in the presence or absence of the class I KDAC inhibitor MS275 (5  $\mu$ M), a putative DUB inhibitor identified by screening (10  $\mu$ M) or vehicle. Expression of four selected proteasomal genes was examined by qPCR. Proteasomal activity was determined by a selective proteasome assay (Promega). MG132, a proteasome inhibitor, was used as a positive control.

**Results:** Pro-inflammatory cytokines significantly induced the expression of the proteasomal subunits, PSMA2 (170%), PSMA7 (212%), PSME1 (429%) and PSMB10 (1050%) in a time-dependent manner ( $n=4$ ,  $p<0.01$ , ANOVA, post hoc t-test). This induction was partially reversed by MS275 and to a lesser extent by the DUB inhibitor ( $n=4$ ,  $p<0.05$ , ANOVA, post hoc t-test). Furthermore, cytokines induced proteasomal activity after one hour persisting in the ensuing twelve hour incubation period (129%, 22 independent wells,  $p<0.01$ , ANOVA, post hoc t-test). Interestingly, this increase in activity was not affected by either KDAC inhibition or DUB inhibition.

**Conclusion:** The expression and activation of the proteasome is induced by pro-inflammatory cytokines in INS-1 cells, but only cytokine-induced gene expression is counteracted by protective small molecules. These data suggest that the protective effects of HDAC and DUB inhibition are not mediated by preventing cytokine-induced proteasomal activity.

*Supported by: NIH Type 1 Diabetes Pathfinder award, BW*

## PS 027 Cytokines and beta cell damage

### 495

#### The Glutamate transporter GLT1/EAAT2: a promising target to arrest beta cell dysfunction and death in diabetes mellitus

A.M. Davalli<sup>1</sup>, E.S. Di Cairano<sup>2</sup>, M. Iaquinto<sup>2</sup>, S. Moretti<sup>2</sup>, C. Bazzini<sup>3</sup>, S. La Rosa<sup>4</sup>, F. Bertuzzi<sup>5</sup>, F. Folli<sup>6</sup>, C. Perego<sup>2</sup>;

<sup>1</sup>Internal Medicine, Diabetes & Endocrinology, San Raffaele Hospital, Milan, Italy, <sup>2</sup>Dept of Molecular Science Applied to Biosystems, University of Milano, Italy, <sup>3</sup>Biomolecular Science and Biotechnology, University of Milano, Italy, <sup>4</sup>Anatomical Pathology, Ospedale di Circolo Varese, Italy, <sup>5</sup>Niguarda Cà Granda Hospital, Milano, Italy, <sup>6</sup>Dept. of Medicine Diabetes Division, University of Texas Health Science Center, USA.

**Background and aims:** The clinical course of Diabetes Mellitus is characterized by a progressive decline in  $\beta$ -cell function and mass. Understanding the causes for  $\beta$ -cell failure and death is therefore of capital importance to develop new and more effective therapeutic strategies. We have recently shown that glutamate, an important signalling molecule in islet of Langerhans, represents a new, previously unknown, insult for  $\beta$ -cells. Noteworthy, increased glutamate levels have been found in sera of T1D and T2D subjects, suggesting that abnormal glutamate homeostasis may be involved in diabetes pathogenesis. Our previous work demonstrates that the glutamate transporter GLT1 is a key regulator of glutamate clearance in the islet and in the control of  $\beta$ -cell integrity and a candidate target for pharmacological intervention. Aim of the proposed research is to verify whether GLT1 dysfunction may play a role in diabetes mellitus pathogenesis.

**Materials and methods:** The localization of GLT1 in human pancreases from 5 healthy subjects (age:  $69\pm7$  years; M/F:3/2) and 8 T2DM patients (age:  $67\pm8$  years; M/F:5/3; disease duration range 1.5–30 years) was analysed by immunohistochemistry. The molecular mechanisms responsible for GLT1 alterations were investigated in human islet preparations exposed for 3 days to metabolic insults (chronic hyperglycaemia 16.7 mM glucose; pro-inflammatory cytokines TNF $\alpha$ , IL $\beta$ , IFN $\gamma$ ) by means of western blotting, uptake and immunofluorescence experiments. Data are expressed as mean value $\pm$ SE. The study has been reviewed by the local ethics committee.

**Results:** In healthy control pancreases GLT1 staining was detected exclusively in the islet, and confined to the plasma membrane of  $\beta$ -cells. In contrast, in 5 of 8 T2DM pancreases GLT1 staining was mainly detected in intracellular compartments. Interestingly the 5 patients showing complete GLT1 internalization were those treated with insulin likely because of secondary failure to the oral antidiabetic agents. When exposed to high glucose, the total GLT1 expression of cultured human islets remained stable ( $94\pm6\%$  of 5.5 mM glucose), however, the transporter translocated from the cell membrane into intracellular vesicular compartments. Accordingly, GLT1 transport activity measured by means of [ $^3$ H]D-glutamate uptake was inhibited by  $25\pm5\%$  as compared to 5.5 mM glucose ( $p<0.05$ ). Three days incubation with pro-inflammatory cytokines alone (100U/ml TNF $\alpha$ ) or in combination (100U/ml TNF $\alpha$ , 50U/ml IL $\beta$ , 100U/ml IFN $\gamma$ ) caused a significant reduction of GLT1 expression ( $65\pm8\%$  and  $53\pm3\%$  as compared to untreated islets, respectively,  $p<0.05$ ).

**Conclusion:** Abnormal GLT1 expression and function may be an early event in diabetes pathogenesis and it may contribute significantly to  $\beta$ -cell loss. Development of drugs targeting GLT1 may represent a novel approach to diabetes treatment.

*Supported by: PUR2009*

## 496

### Activation of 5-HT receptors 2a and 2b promotes viability and potentiates glucose stimulated insulin release in a clonal beta cell (INS 832/13) line

A. Balhuizen, H. Bennet, L. Groop, M. Fex;

Department of Clinical Sciences, Lund University, Malmö, Sweden.

**Background and aims:** The role of serotonin (5-HT) in glucose homeostasis and its effect on insulin secretion is not yet fully understood 5-HT has been shown to modulate insulin secretion from islets of Langerhans either through serotonylation of GTPases or by binding to G-protein coupled receptors; these receptors display either inhibitory or stimulatory effects on



glucose stimulated insulin secretion (GSIS). The serotonin 2 family, signals via Gq, phospholipase-C, and consists of three subtypes: 2a, 2b and 2c. These are all known to have central effects on glucose homeostasis by regulation of food intake and energy expenditure. In peripheral tissues, the 5-HT<sub>2</sub> receptor family has been shown to have proliferative and cyto-protective properties in neurons and cardiomyocytes, respectively. With this notion, we will investigate if signalling through these receptors can protect beta cells against gluco/lipotoxicity and/or cytokine induced cell death.

**Material and methods:** Quantitative (Q)PCR was used to detect receptor expression from mRNA isolated from the (physiologically relevant) clonal beta cells (INS(832/13). Expression was detected with primers for HTR2a and HTR2b and HTR2c and compared against internal reference genes (HPRT, POLR2A and PPIA). The following agonists were used: 5-HT<sub>2a</sub>, TCB-2 (0–500 μM) and for 5-HT<sub>2b</sub> α-Methyl serotonin maleate salt (α-MET) (0–100 μM). INS(832/13) cells were incubated with cytokines: rrIL-1β10 ng/ml, rrTNFα 16 ng/ml and rrIFNγ 7.2 ng/ml or a combination of Palmitate (0.6 mM) and high glucose (30 mM). Insulin concentrations were measured with Mouse Insulin ELISA and protein content with BCA. Apoptotic cells were stained for Annexin-V and Propidium Iodide and detected with a FACS-system. INS(832/13) cell proliferation rate was detected with a Ki-67 antibody and quantified with a FACS-system.

**Results:** INS 832/13-cells express 5-HT<sub>2a</sub> and 5-HT<sub>2b</sub> but not 5-HT<sub>2c</sub> receptors. Long term incubation with agonists TCB-2 and α-MET showed a tendency towards an increase in expression of both receptors, suggesting a regulatory feedback loop on receptor signalling and expression of receptors. Moreover, α-MET and TCB-2, potentiated glucose stimulated insulin secretion in a concentration-dependent manner; TCB-2 displayed an EC<sub>50</sub> of 5.8·10<sup>-6</sup> at 16.7 mM glucose and 100 μM TCB-2 increased GSIS with a p-value of 0.007. In addition, at 16.7 mM glucose, 5 μM α-MET increased GSIS with a p-value of 0.0016. Preliminary data indicates that α-MET and TCB-2 decrease the apoptotic rate of INS-cells incubated with proinflammatory cytokines and during gluco/lipotoxic conditions.

**Conclusion:** Our results show that serotonin receptors 5-HT<sub>2a</sub> and 5-HT<sub>2b</sub> are expressed in INS(832/13) cells and when activated these receptors are able to potentiate glucose stimulated insulin secretion. In addition, α-MET via the 5-HT<sub>2b</sub> receptor display cell protective effects against both proinflammatory cytokines and gluco/lipotoxic conditions. The present study contributes to the understanding how serotonin affects the pancreatic beta cell, and how the 5-HT<sub>2</sub> receptor family may promote beta cell viability and function in type 2 diabetes.

*Supported by: Albert Pahlsson foundation*

## 497

### Galectin-3 deficiency confers resistance to cytokine-induced apoptosis of pancreatic islets - role of mitochondrial pathway

I. Nikolic<sup>1</sup>, T. Saksida<sup>1</sup>, N. Zdravkovic<sup>2</sup>, I. Stojanovic<sup>1</sup>, M.L. Lukic<sup>2</sup>, S. Stosic-Grujicic<sup>1</sup>

<sup>1</sup>Immunology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Serbia, <sup>2</sup>Center for Molecular Medicine, Faculty of Medicine, University of Kragujevac, Serbia.

**Background and aims:** Galectin-3 (Gal-3) is β-galactoside-binding lectin expressed in variety of tissues and cell types and has diverse functions, including promotion of inflammatory response and triggering apoptosis. However, the precise role of Gal-3 in type 1 diabetes remains obscure. Since we have previously shown that Gal-3 deficient (Gal-3<sup>-/-</sup>) mice are relatively resistant to diabetogenesis induced by multiple low doses of streptozotocin, the aim of this study was to examine the effect of Gal-3 deficiency at the level of pancreatic islets in the setting seen during diabetes pathogenesis.

**Materials and methods:** We exposed mouse pancreatic islets isolated from Gal-3<sup>-/-</sup> and WT (C57BL/6) mice by collagenase digestion to proinflammatory cytokines (IL-1β+IFN-γ+TNF-α, 10 ng/ml each) and analysed their survival. In addition, 30 minutes before adding cytokines islets from WT mice were incubated in the presence or absence of chemical Gal-3 inhibitors: chemical inhibitor (Td131\_1, 10 mM) or natural competitor β-D-lactose monohydrate (50 mM). After incubation, β-cell apoptosis was measured by histone-DNA ELISA. Expression profiles of various related genes were determined at mRNA and protein levels by real time PCR (RT-PCR) and Western blot, respectively.

**Results:** Deficiency of Gal-3 either hereditary or induced through application of inhibitors promotes better survival of pancreatic islets after apoptotic stimulus. We further analysed molecular mechanisms involved in protection of Gal-3<sup>-/-</sup> islets from cytokine-induced apoptosis. The lack of Gal-3 impairs

activation of caspase-3 after cytotoxic stimulus. Protein level of activated caspase-3 after cytotoxic stimulus was lower in Gal-3<sup>-/-</sup> islets compared to WT islets. Also, Gal-3 deletion alters the pattern of expression of pro- and anti-apoptotic molecules determined by RT-PCR analysis of stimulated islets. mRNA levels of anti-apoptotic molecules Bcl-2 and Bcl-X<sub>L</sub> were up-regulated in Gal-3<sup>-/-</sup> islets compared to WT islets, while there was no difference in expression of pro-apoptotic Bax. Western blot analysis confirmed this shift of pro- and anti-apoptotic molecules in favour of anti-apoptotic molecules after cytotoxic stimulus. Furthermore, preserved viability of Gal-3<sup>-/-</sup> islets coincided with up-regulation of phospho-ERK1/2, a pro-survival kinase.

**Conclusion:** This study suggests that Gal-3 is involved in pancreatic islets apoptosis in the inflammatory milieu that occurs during diabetes pathogenesis and implicates impairment of mitochondrial apoptotic pathway as a key event in protection from beta cell apoptosis in the absence of Gal-3.

*Supported by: Serbian Ministry of Education and Science (Grants No.: 173013 and 175069)*

## 498

### Cytoprotective effect of prostacyclin against cytokine toxicity in insulin-secreting cells depends on the activation of its extracellular receptors

E. Gurgul-Convey, K. Hanzelka, S. Lenzen;

Institute of Clinical Biochemistry, Hannover Medical School, Germany.

**Background and aims:** The overexpression of prostacyclin synthase (PGIS), the enzyme synthesizing the antiinflammatory prostaglandin I<sub>2</sub> (prostacyclin), has been shown to protect pancreatic beta cells against cytokine toxicity via inhibition of the cytokine-mediated NFκB activation and nitrosative stress. It remains unknown if prostacyclin produced by PGIS acts only within or if it must be released from the cell of origin and act via the activation of its extracellular receptors. Therefore the aim of this study was to analyze the fashion of the action of prostacyclin in insulin-secreting cells.

**Materials and methods:** Insulin-secreting INS1E cells were stably transfected either with an empty pcDNA3.1 vector (INS1E-control) or with the pcDNA3.1-hPGIS (INS1E-PGIS). Cells were treated with IL-1β 600 U/ml or with a cytokine mix (IL-1β 60 U/ml, TNFα 185 U/ml, IFNγ 14 U/ml) in the absence or presence of a stable prostacyclin analogue (iloprost) or the prostacyclin receptor blocker (10 nM CAY10441) for 24 h. Thereafter proliferation was measured by a BrdU ELISA, caspase-3 by flow cytometry, cAMP by ELISA, NFκB and iNOS-promoter by SEAP reporter gene assays, nitrite by Griess method and ATP by ATPlite assay.

**Results:** INS1E-PGIS cells had a significantly higher content of ATP in comparison with INS1E-control cells (5.7 vs. 2.8 nmol/mg protein). Cytokines reduced ATP content in INS1E-control cells (IL-1β 50%, cytokine mix: 75% vs. 100% untreated). This reduction was counteracted by iloprost and PGIS overexpression and enhanced by CAY10441. The cAMP content in INS1E-control cells was 13±1, while in INS1E-PGIS cells 22±2 pmol/mg protein. CAY10441 reduced the cAMP content in INS1E-PGIS cells (9±1 pmol/mg protein). IL-1β and a cytokine mix induced caspase-3 activation in INS1E-control cells (IL-1β: 151%, cytokine mix: 258% vs. 100% untreated). Iloprost inhibited and PGIS overexpression prevented this activation (IL-1β: 81%, cytokine mix: 118% vs. 100% untreated), and this was reversed by CAY10441. The basal proliferation rate of INS1E-PGIS cells was significantly higher as in INS1E-control cells (140 vs. 100% in INS1E-control cells). Cytokines reduced proliferation of INS1E-control cells (IL-1β 60%, cytokine mix: 70% vs. 100% untreated). The cytokine-mediated inhibition of cell proliferation was weaker in INS1E-PGIS cells, but was enhanced by CAY10441. PGIS overexpression protected also against cytokine-stimulated NFκB activation, iNOS promoter induction and nitrite formation (INS1E-control NFκB: IL-1β 7-fold, cytokine mix 5-fold; INS1E-PGIS: IL-1β 2-fold, cytokine mix: 3-fold; INS1E-control iNOS promoter IL-1β 8-fold, cytokine mix 10-fold; INS1E-PGIS: IL-1β 3.5-fold, cytokine mix 4-fold; INS1E-control: nitrite IL-1β 37, cytokine mix 100; INS1E-PGIS: IL-1β 7, cytokine mix 14 pmol/μg protein). This protection was reduced by CAY10441.

**Conclusion:** The prostacyclin analogue and PGIS overexpression protected insulin-secreting cells against cytokine toxicity, and this cytoprotective effect correlated with an increased content of ATP and cAMP. The protection provided by prostacyclin strongly, but not exclusively, depends on the prostacyclin-induced activation of the extracellular prostacyclin receptors. Thus chemical prostacyclin analogues may be drug candidates for protection of beta cells against cytokine toxicity in type 1 diabetes.

*Supported by: ZKK*

## 499

**The adipocytokines Nampt and vaspin have no effect on beta cell survival but potentiate glucose stimulated insulin secretion**

K. Stolz<sup>1</sup>, R. Spinnler<sup>2</sup>, T. Gorski<sup>2</sup>, S. Lau<sup>2</sup>, S. Schuster<sup>2</sup>, A. Garten<sup>2</sup>, J. Kratzsch<sup>3</sup>, P. Kovacs<sup>4</sup>, J. Heiker<sup>5</sup>, A. Beck-Sickinger<sup>6</sup>, E. de Koning<sup>7</sup>, A. Körner<sup>2</sup>, W. Kieß<sup>2</sup>, K. Maedler<sup>1</sup>;

<sup>1</sup>Centre for Biomolecular Interactions, University of Bremen, <sup>2</sup>University Hospital for Children and Adolescents, Center for Pediatric Research Leipzig (CPL), <sup>3</sup>Institute of Laboratory Medicine, Leipzig, <sup>4</sup>Interdisciplinary Centre for Clinical Research, Leipzig, Germany, <sup>5</sup>Department of Internal Medicine, Leipzig, Germany, <sup>6</sup>Institute of Biochemistry, Leipzig, <sup>7</sup>Leiden University Medical Center, Leiden, Netherlands.

**Background and aims:** Obesity is associated with a dysregulation of beta-cell and adipocyte function. The molecular interactions between adipose tissue and beta-cells are not yet fully elucidated. We investigated, whether the newly identified adipocytokines Nampt and vaspin, which all have been independently associated with obesity and T2DM directly influence beta-cell survival and function.

**Materials and methods:** The effect of the adipocytokines on viability of INS-1E cells was assessed by WST-1 assay. Apoptosis was measured by Annexin V/PI, TUNEL assay or by activated caspase-3. Adenylate kinase release was determined to assess cytotoxicity. Chronic and acute effects of the adipocytokines on insulin secretion were assessed by glucose stimulated insulin secretion in human islets.

**Results:** While stimulation of beta-cells with the cytokines IL-1 $\beta$ , TNF $\alpha$  and IFN- $\gamma$  or palmitate significantly decreased viability, Nampt and vaspin showed no direct effect on viability in INS-1E cells or in human islets, neither alone nor in presence of pro-diabetic conditions (elevated glucose concentrations and palmitate or cytokines). At chronic conditions over 3 days of culture, Nampt, its enzymatic product nicotinamide Mononucleotide (NMN) and vaspin had no effects on insulin secretion. In contrast, both adipocytokines potentiated glucose stimulated insulin secretion acutely during 1h incubation of human islets.

**Conclusion:** Nampt and vaspin did not influence beta-cell viability nor apoptosis but acutely potentiated glucose stimulated insulin secretion.

*Supported by: DFG*

## 500

**Sodium tungstate promotes beta cell survival in IRS2-deficient mice model**

J. Oliveira<sup>1,2</sup>, S. Rebuffat<sup>1,2</sup>, A. García<sup>1,2</sup>, A. Novials<sup>1,2</sup>, R. Gasa<sup>1,2</sup>, R. Gomis<sup>1,2</sup>;

<sup>1</sup>IDIBAPS/Hospital Clinic, <sup>2</sup>Ciberdem, Barcelona, Spain.

The control of pancreatic  $\beta$ -cell growth and survival plays an essential role in the pathogenesis of type 2 diabetes. The failure of  $\beta$ -cell mass to adequately expand in settings of insulin resistance results in the appearance of glucose intolerance and type 2 diabetes. The molecular mechanisms involved in this failure are poorly known. Nevertheless, it is well accepted that signal transduction via Insulin/Insulin growth factor/IRS-2 is critically involved in this process. Indeed, the ablation of the IRS-2 gene in mice results in impaired  $\beta$ -cell survival and the subsequent development of a phenotype with characteristics of type-2 diabetes. Prior work by our group has described sodium tungstate as a possible pharmacological modulator of  $\beta$ -cell mass. Here, we investigated if sodium tungstate administration could reverse the diabetic phenotype of IRS-2 knockout mice. 10-week old wild type (WT) and knockout IRS2 C57Bl/6 (Irs2<sup>-/-</sup>) mice were given a solution of 2mg/ml of sodium tungstate (treated groups) or distilled water (untreated groups) for 21 days. During this period, basal and postprandial blood glucose levels were recorded.  $\beta$ -cell mass was quantified using immunohistochemical and morphometric analysis. Apoptotic cell death was determined by cleaved caspase 3 staining. Global gene expression profiles were analyzed in isolated pancreatic islets using mouse genome Affymetrix arrays and protein expression was assessed using Panorama Cell signaling Antibody arrays. The administration of sodium tungstate significantly decreased basal glycemia (from 221 $\pm$ 26 to 134 $\pm$ 23 mg/dl, n=9) and improved glucose tolerance in Irs2<sup>-/-</sup> mice relative to untreated Irs2<sup>-/-</sup> while no changes were observed in WT animals. As expected, untreated Irs2<sup>-/-</sup> mice showed pronounced hyperglycaemia and reduced  $\beta$ -cell mass (0.28 $\pm$ 0.2 vs 0.94 $\pm$ 0.2 mg, p<0.001, untreated Irs2<sup>-/-</sup> vs WT) owing to smaller size islets containing fewer  $\beta$ -cells. Remarkably, the treatment with tungstate prevented the loss of  $\beta$ -cell mass in Irs2<sup>-/-</sup> mice (0.61 $\pm$ 0.3 mg vs 0.28 $\pm$ 0.2 mg, p<0.05, treated vs untreated Irs2<sup>-/-</sup>), which correlated

with decreased  $\beta$ -cell apoptosis (2.6%  $\pm$ 0.01 vs 17.2%  $\pm$ 0.14, p<0.05, treated vs untreated Irs2<sup>-/-</sup>) and increased  $\beta$ -cell proliferation (0.45% $\pm$ 0.0084 vs 0.01% $\pm$ 0.0001). These data was supported by transcriptomic analysis of isolated pancreatic islets that revealed downregulation of several apoptosis and inflammation-related genes (such as Gasdermin and Cidea) in treated Irs2<sup>-/-</sup> relative to untreated Irs2<sup>-/-</sup> mice. Interestingly, 30% of these genes were upregulated in the Irs2<sup>-/-</sup> untreated group and downregulated by tungstate treatment. Furthermore, protein expression analysis confirmed the inhibition of cellular apoptosis pathways. Specifically, Fas (CD95/Apo-1) and FADD proteins, which are involved in death receptor induced apoptosis, and caspase 9, an initiator caspase linked to the mitochondrial death pathway, were found to be inhibited by tungstate treatment in Irs2<sup>-/-</sup> mice. Our findings demonstrate that sodium tungstate treatment ameliorates the diabetic phenotype of IRS-2 knockout mice, likely through inhibition of  $\beta$ -cell loss by repressing the apoptotic machinery. Further experiments are currently being performed in order to unravel the molecular mechanisms underlying the effects of tungstate on  $\beta$ -cell survival.

*Supported by: MICINN; Generalitat de Catalunya*

## PS 028 Models of beta cell failure in type 2 diabetes

501

### Non-invasive in-vivo analysis of beta cell function and mass by MRI

A. Meyer<sup>1</sup>, E. Kuestermann<sup>2</sup>, K. Stolz<sup>1</sup>, J. Bergemann<sup>1</sup>, W. Dreher<sup>2</sup>, K. Maedler<sup>1</sup>;

<sup>1</sup>Centre for Biomolecular Interactions, <sup>2</sup>FB2, MR imaging & in-vivo spectroscopy, University of Bremen, Germany.

**Background and aims:** Diabetes diagnostic, therapy and research would strongly benefit from non-invasive methods of analyzing pancreatic  $\beta$ -cell mass (BCM). Besides BCM, the capacity of the  $\beta$ -cell to produce sufficient amounts of insulin depends on  $\beta$ -cell function. Thus, we aimed to develop a strategy to monitor the functional  $\beta$ -cell mass by measuring manganese (Mn2+) uptake into  $\beta$ -cells by T1-weighted in-vivo Magnet Resonance Imaging (MRI). Divalent manganese ions (Mn2+), like Ca2+, enter metabolically active  $\beta$ -cells glucose-dependently through voltage-gated calcium channels, which open in response to a rise in extracellular glucose and the subsequent closure of ATP-sensitive K+ channels.

**Materials and methods:** C57Bl/6J mice were fed a normal diet (ND) or high fat/high sucrose diet (HFD) over 6 weeks. Glucose tolerance was monitored by intraperitoneal glucose tolerance tests and classical measures of  $\beta$ -cell mass in isolated pancreases were performed. For MRI imaging, 2 g/kg Glucose was injected i.p., followed by intravenous MnCl2 (i.v.). Respiratory-triggered 3D-“Snapshot FLASH” images were obtained using a 7T Bruker Biospec system (70/20 USR) with inversion recovery preparation for kinetic measurements of the Mn2+-uptake. The scan-TR was 6 s to ensure full relaxation and to avoid any breathing rate dependent T1-effects. Pancreas signal was normalized to the final liver signal.

**Results:** The comparison of temporal signal changes upon i.v. injection of manganese ions reveals strong differences between ND and HFD fed mice. During the first 2 weeks of the diet a 1.3-fold higher Mn2+-signal could be detected in the pancreas of the HFD compared to the ND group, accordingly, also glucose tolerance was improved in the HFD fed mice. After 3 weeks of diet, comparable MRI signals were detected in both groups together with a similar glucose tolerance. After 6 weeks of diet the signal intensity of the pancreas in the HFD was 1.4-fold lower than in the ND group ( $p < 0.05$ ). At the same time, glucose tolerance as well as insulin secretion during the ipGTT was impaired, while  $\beta$ -cell mass was 1.5-fold increased in the HFD group ( $p < 0.05$ ), indicating the compensatory growth of  $\beta$ -cells in response to the HFD after 6 weeks. These data show, that MRI using Mn2+ analyses the functional rather than the total  $\beta$ -cell mass. No long-term effects of Mn2+ on glucose tolerance, even after multiple injections were observed.

**Conclusion:** Our optimized protocol for T1-weighted imaging of the mouse abdomen fulfills the requirements of non-invasive MR imaging. The analysis of glucose-dependent Mn2+-uptake from the mouse pancreas is a technique, which can be used in-vivo to assess the functionality of both grafted and endogenous pancreatic islets.

Supported by: FP7

502

### Lysosomal acid lipase and lipophagy are constitutive negative regulators of glucose stimulated insulin secretion from pancreatic beta cells

G. Pearson<sup>1</sup>, N. Mellett<sup>2</sup>, P. Bourbon<sup>3</sup>, C. Cosner<sup>3</sup>, P. Helquist<sup>3</sup>, P. Meikle<sup>2</sup>, T. Biden<sup>1</sup>;

<sup>1</sup>Garvan Institute of Medical Research, Sydney, Australia, <sup>2</sup>Baker IDI, Melbourne, Australia, <sup>3</sup>University of Notre Dame, USA.

**Background and aims:** Glucose stimulated insulin secretion (GSIS) involves both initiation and amplification pathways. Lipolytic breakdown of endogenous lipid pools contributes to the amplification phase, mediated by acute activation of neutral lipases. Chronic downregulation of autophagy (where macromolecules are delivered to the lysosome for degradation) also inhibits insulin secretion. More recently, it has been shown in other cell types, that endogenous lipid can be metabolised by autophagy (lipophagy) using lysosomal acid lipase (LAL). Our current aim was to investigate LAL in pancreatic beta cells, and its role in lipophagy and GSIS.

**Methods:** MIN6 cells were incubated for 24h with <sup>14</sup>C-palmitic acid, followed by glucose stimulation (2- or 20mmol/l glucose for 1h), and then subjected

to subcellular fraction to determine lipid localisation. GSIS was measured by insulin RIA in: MIN6 cells treated for 24h with 5 $\mu$ mol/l lalistat (LAL inhibitor); MIN6 cells transfected with atg7 siRNA; and mouse islets treated for 24h with 5mmol/l 3-methyladenine (3MA). Lipid composition in lalistat-treated MIN6 cells was measured using lipid GC-MS. Gene and protein expression changes were assessed with RT-PCR and Western blot. Statistical analysis was by one-way ANOVA with bonferroni post-test, unless otherwise stated.

**Results:** Incorporation of <sup>14</sup>C-palmitic acid into MIN6 cells showed that 15% of the total labelled lipid was localised to lysosomal fractions. RT-PCR and Western blot analysis showed abundant expression of LAL in MIN6 cells, mouse and human islets. GSIS was upregulated 1.8 fold in MIN6 cells pre-treated with lalistat (ng insulin/ug protein/h $\pm$ SEM: control 6.08 $\pm$ 0.53; lalistat 11.04 $\pm$ 0.58  $n=3$ ;  $P < 0.001$ ). Lalistat treatment of MIN6 cells increased neutral lipids (nmol/mg protein $\pm$ SEM,  $n=4$ ): cholesterol ester 6.7 fold (control 13.6 $\pm$ 2.62; lalistat 92.1 $\pm$ 11.4;  $P < 0.01$  t-test), triacylglycerol 1.7 fold (control 0.69 $\pm$ 0.12; lalistat 1.15 $\pm$ 0.14;  $P < 0.05$  t-test) and diacylglycerol 2 fold (control 5.71 $\pm$ 1.23; lalistat 11.8 $\pm$ 1.58;  $P < 0.05$  t-test). Diacylglycerol was further increased, approximately 2 fold ( $P < 0.01$  t-test), with 20mmol/l glucose after lalistat treatment. Inhibiting autophagy, with atg7 siRNA in MIN6 cells, resulted in a 1.5 fold increase in GSIS (ng insulin/ug protein/h $\pm$ SEM: control si 1.52 $\pm$ 0.12; atg7 si 2.26 $\pm$ 0.21  $n=3$ ;  $P < 0.01$ ). In mouse islets inhibiting autophagy with 3MA caused a 2 fold increase in GSIS (pg insulin/islet/h $\pm$ SEM: control 486.1 $\pm$ 199.1; 3MA 953.4 $\pm$ 230  $n=4$ ;  $p < 0.05$  paired t-test).

**Conclusion:** Our data suggests that lysosomal breakdown of lipids, via the action of LAL and potentially involving lipophagy, is a constitutive negative regulator of GSIS. This process could be mediated by depletion of substrate for the neutral lipases that are activated by glucose.

Supported by: NHMRC of Australia



## PS 028 Models of beta cell failure in type 2 diabetes

503

### Characterisation of pancreatic islets from two lines of selectively bred mice with different susceptibilities to high fat diet-induced glucose intolerance

M. Nagao, A. Asai, M. Kawahara, Y. Sato, Y. Nakajima, H. Sugihara, S. Oikawa;

Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, Tokyo, Japan.

**Background and aims:** Several animal models are currently used in the research field of type 2 diabetes. However, they could not explain the genetic factors for determining the susceptibility to diet-induced diabetes. We recently established two lines of mice with different susceptibilities (prone and resistant) to high fat diet (HFD)-induced glucose intolerance by selective breeding (designated SDG-P and SDG-R, respectively). Even before HFD feeding, SDG-P mice showed modestly higher blood glucose levels in OGTT relative to SDG-R mice. After receiving HFD, the glucose intolerance of SDG-P mice became more evident without hyperinsulinemia. In this study, we evaluated morphology and function of pancreatic islets from both mouse lines to investigate the heritable factors for determining the susceptibility to diet-induced glucose intolerance.

**Materials and methods:** Male mice of each strain fed HFD (32% energy as fat) for 5 weeks (from 5 to 10 weeks of age). Before and after HFD feeding (SDG-P; n=9, SDG-R; n=9, respectively), islets were isolated by collagenase perfusion. Islet morphology was evaluated under a stereomicroscope. Glucose- and KCl- induced insulin secretion (GSIS and KSIS) from the isolated islets were assessed *ex vivo* by batch incubation in media containing 16.7 mmol/l glucose and 34.8 mmol/l KCl, respectively. Gene expressions related to insulin secretion in the isolated islets were evaluated by real time quantitative PCR. All data are expressed as means  $\pm$  SEM. Mean values were compared using Student's *t* test.

**Results:** Before HFD feeding, more numbers of islets were yield from SDG-P mice than SDG-R mice (SDG-P vs. SDG-R;  $173 \pm 19$  vs.  $106 \pm 17$  islets/mouse,  $P=0.020$ ), whereas apparent islet size was not different ( $22.6 \pm 1.0$  vs.  $21.9 \pm 1.0 \times 10^3 \mu\text{m}^2/\text{islet}$ ,  $P=0.61$ ). After HFD feeding, no significant differences were observed in the number of isolated islets between the two strains ( $224 \pm 24$  vs.  $183 \pm 14$  islets/mouse,  $P=0.15$ ); however, apparent islet size of SDG-P mice became larger than that of SDG-R mice ( $31.7 \pm 1.2$  vs.  $22.4 \pm 0.6 \times 10^3 \mu\text{m}^2/\text{islet}$ ,  $P<0.001$ ). Regardless of whether it was before or after HFD feeding, GSIS and KSIS from the isolated islets from SDG-P mice were significantly lower than those from SDG-R mice. Before HFD feeding, GSIS and KSIS of SDG-P islets were  $43.9 \pm 4.3\%$  ( $P=0.024$ ) and  $63.4 \pm 4.9\%$  ( $P=0.007$ ) of SDG-R islets, respectively; after HFD feeding, they were  $45.2 \pm 3.6\%$  ( $P=0.001$ ) and  $45.8 \pm 2.8\%$  ( $P<0.001$ ), respectively. In addition, mRNA expression levels of GLUT-2 (before HFD, after HFD;  $P=0.024$ ,  $P=0.023$ ), PDX-1 ( $P=0.023$ ,  $P=0.014$ ), and Syntaxin-1a ( $P=0.059$ ,  $P=0.028$ ) were lower in SDG-P islets as compared with SDG-R ones both before and after the HFD feeding.

**Conclusion:** The HFD-induced glucose intolerance prone SDG-P mice showed spontaneous impairments of GSIS and KSIS in pancreatic islets. Although, hypertrophy was observed after HFD feeding in SDG-P islets, the compensation by beta-cell proliferation was insufficient to regulate glucose metabolism. Results of real time PCR analysis suggest that reduced gene expressions of glucose sensing, exocytosis, and transcription factor in SDG-P islets contribute to the insufficiency of insulin secretion and beta-cell proliferation. Taken together, these characteristics in pancreatic islets may determine the susceptibility to HFD-induced glucose intolerance.

504

### Membrane phospholipid remodelling under glucolipotoxic conditions promotes beta cell dysfunction

S. Sasson<sup>1</sup>, C. Ferreri<sup>2</sup>, C. Chatgililoglu<sup>3</sup>, N. Kaiser<sup>4</sup>, G. Cohen<sup>1</sup>;

<sup>1</sup>Pharmacology, Hebrew University Faculty of Medicine, Jerusalem, Israel,

<sup>2</sup>ISOF-BioFreeRadicals, Bologna, Italy, <sup>3</sup>SOF-BioFreeRadicals, Bologna, Italy,

<sup>4</sup>Endocrinology & Metabolism Service, The Hebrew University Hadassah Medical Center, Jerusalem, Israel.

**Background and aims:** Hyperglycemia and high fatty acids concentrations (*i.e.*, palmitic acid, PA, 16:0) are detrimental to  $\beta$ -cells. The combination of

both deteriorates  $\beta$ -cell function in a phenomenon termed glucolipotoxicity. Analysis of membrane phospholipids in INS-1E  $\beta$ -cells revealed a significant glucose-induced changes in the content of various saturated (SFA)-, mono-unsaturated (MUFA)- and poly-unsaturated fatty acids (PUFA). Of a particular interest is the release of arachidonic (AA, 20:4) and linoleic acids (LA, 18:2) into the cell interior. Both fatty acids undergo free radical-induced peroxidation and generate 4-hydroxynonenal (4-HNE), which at high levels becomes cytotoxic. The present study aimed at elucidating the contribution of phospholipid remodeling to glucolipotoxic interactions in  $\beta$ -cells.

**Materials and methods:** INS-1E cells were maintained at 5, 11 and 25 mM glucose for 48h, without or with PA (50–500  $\mu\text{M}$ ) added during the last 16h of incubation. The cells were then harvested, extracted and chemically-formed methyl-esters of fatty acids were analyzed by gas-chromatography. In parallel, glucose-stimulated insulin secretion (GSIS) and cell viability assays were performed. Western blot analysis of 4-HNE-histidine adducts was employed to estimate cell-associated 4-HNE.

**Results:** As expected, exposure of INS-1E cells to PA increased concentration-dependently its content in phospholipids. Concomitantly, an enhanced desaturation of PA to palmitoleic acid (16:1) increased the abundance of the latter in phospholipids. Otherwise, other glucose- and PA-induced changes in the abundance of fatty acids in phospholipids were additive. Of interest is the finding that PA significantly and concentration-dependently reduced AA and LA content in phospholipids, with maximal effects ( $\sim 40\%$  reduction) observed already after 2h incubation in the presence of 50–125  $\mu\text{M}$  PA at all glucose levels. Cell viability was not affected under these conditions and GSIS was nearly 30–40% higher than in the control cells. Cytotoxicity was apparent only in cells maintained at 11 or 25 mM glucose and exposed to 250–500  $\mu\text{M}$  of PA for 12–16h: the cell number and total cell insulin content were reduced by  $\sim 40\%$  while GSIS was blunted. There was a strong correlation between the generation of 4-HNE and these cytotoxic interactions. Interestingly, no such detrimental effects of PA were observed in cells maintained 5 mM glucose, in which 4-HNE level was moderate. Finally, a careful examination of the lipidomic analysis data showed additional glucose- and PA-induced changes in the content of various  $\omega$ -3- and  $\omega$ -6-PUFA, MUFA and SFA.

**Conclusion:** This study highlights the crucial role of glucose- and fatty acids-induced phospholipid remodeling in regulating  $\beta$ -cell function and in promoting cytotoxicity. It examined the impact of the liberation of AA and LA from phospholipids into the cell interior and the generation of the potent cytotoxic agent 4-HNE. Our current findings accentuate the need for thorough investigations of the mechanism of phospholipid remodeling, the subsequent generation of myriad lipid mediators and their impact on  $\beta$ -cell functions and dysfunction.

Supported by: Israel Science Foundation

505

### Phenotypic characterisation of diabetic INS<sup>C94Y</sup> transgenic pigs

S. Renner<sup>1</sup>, C. Braun<sup>1</sup>, A. Blutke<sup>2</sup>, N. Herbach<sup>2</sup>, A. Wünsch<sup>1</sup>, M. Kurome<sup>1</sup>, B. Kessler<sup>1</sup>, S. Krebs<sup>3</sup>, O. Puk<sup>4</sup>, H. Nagashima<sup>5</sup>, J. Graw<sup>4</sup>, H. Blum<sup>3</sup>, R. Wanke<sup>2</sup>, E. Wolf<sup>1</sup>;

<sup>1</sup>Ludwig-Maximilians Universität München, Oberschleißheim, Germany,

<sup>2</sup>Ludwig-Maximilians Universität München, Institute of Veterinary Pathology, Munich, Germany, <sup>3</sup>Gene Center of the Ludwig Maximilians Universität München, Munich, Germany, <sup>4</sup>Helmholtz Center Munich-German Research Center for Environmental Health, Institute of Developmental Genetics, Neuherberg, Germany, <sup>5</sup>Meiji University, Laboratory of Developmental Engineering, Kawasaki, Japan.

**Background and aims:** Islet (xeno-)transplantation as well as preclinical testing of novel therapeutic strategies require diabetic large animal models showing close physiological and anatomical similarities to humans, *e.g.* pigs. To date, diabetes in the pig can be induced either by pancreatectomy or chemical agents, *e.g.* streptozotocin. Due to substantial drawbacks of these existing models, our aim was to generate a transgenic diabetic pig model, expressing a mutant porcine insulin gene (INS<sup>C94Y</sup>) resembling the human and murine INS<sup>C96Y</sup> mutation which leads to permanent neonatal diabetes.

**Materials and methods:** INS<sup>C94Y</sup> transgenic (tg) pigs were generated by somatic cell nuclear transfer using porcine fetal fibroblasts stably transfected with a porcine INS<sup>C94Y</sup> expression vector (provided by Dr. H. Flawinkel). The ratio of INS<sup>C94Y</sup> and INS transcripts in pancreatic tissue was determined by next generation sequencing technology. The total  $\beta$ -cell volume was determined by quantitative-stereological analyses.

**Results:** In total, 7 INS<sup>C94Y</sup> tg founder boars were born. One founder showed elevated fasting blood glucose levels at the age of 85 days, further increasing

over time. The ratio of *INS*<sup>C94Y</sup> to *INS* transcripts (0.751) in pancreas of the diabetic founder was at least 5-fold higher than in the 6 non-diabetic founders. Under insulin treatment, the diabetic boar developed nearly normal and was successfully mated to non-transgenic sows. The transgene was inherited to 50% of the offspring according to mendelian rules. The *INS*<sup>C94Y</sup> to *INS* transcript ratio in pancreas of F1-offspring was similar to the founder boar (0.775 ± 0.05; n = 3). Transgenic F1-/F2-offspring exhibited significantly elevated random blood glucose levels within 24 hours after birth vs. controls (194 ± 12 vs. 146 ± 9 mg/dl; p=0.03). Fasting insulin levels were unaltered in 8-day-old *INS*<sup>C94Y</sup> tg pigs, but were significantly reduced at the age of 4.5 months vs. controls (2.0 ± 0.42 vs. 5.1 ± 0.77 µU/ml; p<0.01). Also, 4.5-month-old *INS*<sup>C94Y</sup> tg pigs revealed a significantly elevated HOMA-IR index (2.4 ± 0.48 vs. 1.2 ± 0.24; p<0.05) indicating insulin resistance. Despite the early onset of hyperglycemia, total β-cell volume of 8-day-old tg pigs was not different compared to controls (136 ± 37 vs. 137 ± 15 mm<sup>3</sup>; p=0.68). In contrast, 4.5-month-old *INS*<sup>C94Y</sup> tg pigs revealed a 77% reduced total β-cell volume related to BW (4 ± 0.45 vs. 17.2 ± 2.4 mm<sup>3</sup>/kg; p<0.0001). Electron microscopy of β-cells from 4.5-month-old *INS*<sup>C94Y</sup> tg pigs showed a very low number of insulin secretory granules and severe dilation of the endoplasmic reticulum (ER) while in 8-day-old tg piglets insulin granules were present and the ER-dilation less severe.

**Conclusion:** *INS*<sup>C94Y</sup> tg pigs exhibit a stable diabetic phenotype and survive under insulin treatment and are therefore a valuable animal model for numerous applications. The evaluation for the development of diabetic complications is currently ongoing.

**Supported by:** DHFD, Leading-Edge Cluster m4 – Personalized Medicine and Targeted Therapie

## 506

**Reduction in pancreatic beta cell mass caused by enhanced expression of *Cdkn1c* as a result of interaction between C/EBPβ and epigenetic control**  
K. Teruyama<sup>1</sup>, S.-I. Asahara<sup>2</sup>, H. Inoue<sup>1</sup>, H. Etoh<sup>1</sup>, T. Matsuda<sup>2</sup>, S. Seino<sup>2</sup>, Y. Kido<sup>1,2</sup>

<sup>1</sup>Kobe University Graduate School of Health Sciences, <sup>2</sup>Kobe University Graduate School of Medicine, Kobe, Japan.

**Background and aims:** The relationship between pancreatic β-cell mass and the onset and progression of type 2 diabetes mellitus has received a lot of attention in recent years. Our research focused on the *Cdkn1c* gene, which codes for a cell cycle inhibitor, because it is involved in the regulation of pancreatic β-cell mass. The *Cdkn1c* gene is an imprinting gene whose expression is controlled by the non-coding RNA Kcnq1ot1. We found that when the expression of Kcnq1ot1 decreased, epigenetic alterations induced an increase in the expression of *Cdkn1c*, which, in turn, led to a decrease in the pancreatic β-cell mass. We have already reported that the transcription factor C/EBPβ accumulates in the pancreatic β-cells of high fat-fed mice. It has been reported that a binding motif of C/EBPβ is present in the *Cdkn1c* promoter. On the basis of these findings, we hypothesize that the accumulation of C/EBPβ in pancreatic β-cells with reduced expression of Kcnq1ot1 causes further enhancement of *Cdkn1c* expression and promotes pancreatic β-cell failure.

**Materials and methods:** C/EBPβ was overexpressed in MIN6 cells treated with an inhibitor of epigenetic modification. In addition, Kcnq1ot1-truncated C/EBPβ-overexpressing mice (*KT* mice) were produced by cross-breeding Kcnq1ot1-truncated mice with pancreatic β cell-specific C/EBPβ-overexpressing mice.

**Results:** The findings showed that the expression of *Cdkn1c* in MIN6 cells was not influenced by the expression level of C/EBPβ, however, it was enhanced by treatment with an inhibitor of epigenetic modification, and was further enhanced by C/EBPβ overexpression. Studies using chromatin immunoprecipitation (ChIP) assays have shown that the binding between C/EBPβ and the *Cdkn1c* promoter was significantly enhanced during treatment with the inhibitor than under normal conditions. *In vivo* studies have shown that compared to C/EBPβ-overexpressing mice and wild-type mice, *KT* mice show significantly higher blood glucose levels, smaller pancreatic β-cell mass, and enhanced expression of *Cdkn1c* in the pancreatic islets.

**Conclusion:** The findings suggest that the introduction of epigenetic modifications into MIN6 cells or pancreatic β-cells may lead to enhanced binding of C/EBPβ to the *Cdkn1c* promoter, then an increase in the expression levels of *Cdkn1c*, and a decrease in pancreatic β-cell mass.

## 507

**Human Krüppel-like factor 11 differentially regulates human insulin promoter activity in beta cells and non-beta cells via p300, PDX1 and the A3 element**

N. Perakakis<sup>1</sup>, D. Danassi<sup>1</sup>, M. Alt<sup>1</sup>, E. Tsaroucha<sup>1</sup>, A.E. Mehana<sup>1,2</sup>, N. Rimmer<sup>1</sup>, K. Laubner<sup>1</sup>, H. Wang<sup>3</sup>, C.B. Wollheim<sup>4</sup>, J. Seufert<sup>1</sup>, G. Paeth<sup>1</sup>; <sup>1</sup>Endocrinology and Diabetology, Internal Medicine II, University Hospital of Freiburg, Germany, <sup>2</sup>Institute of Biology II, University of Freiburg, Germany, <sup>3</sup>F. Hoffmann-La Roche AG, Metabolic and Vascular Diseases, Basel, Switzerland, <sup>4</sup>Department of Cell Physiology and Metabolism, University of Geneva, Switzerland.

**Objective:** Human Krüppel-like factor 11 (hKLF11) belongs to the Sp1/KLF transcription factor family and has been identified as the MODY7 gene. The molecular functions of hKLF11 in pancreatic beta cells, however, are not well characterised, as it has been reported to both activate and inhibit human proinsulin gene promoter (hInsP) activity. Since hKLF11 can differentially regulate MAO B gene activity depending on recruited cofactors, we investigated possible interactions between hKLF11 and important beta cell transcription factors focusing on the role of the p300/PDX1 transactivation complex. In order to differentiate between endogenous beta cell specific and non-specific effects, we performed our experiments both in beta cells and non-beta cells (HEK293).

**Results:** By mammalian two hybrid assay, we detected direct protein interactions between hKLF11 and p300 but not with PDX1. Cotransfection of hKLF11 and hInsP-driven secreted alkaline phosphatase reporter vectors (-881+54hInsP-SEAP) characterised hKLF11 as an inhibitor of hInsP activity in beta cells. In contrast, we observed strong hKLF11-mediated stimulation of hInsP in HEK293 cells, even in the presence of cotransfected beta-cell-specific PDX1. Both inhibition and stimulation depended on the activity of the coactivator p300, since additional coexpression of hp300 in INS-1E or of the p300 inhibitor E1A in HEK293 neutralised hKLF11 functions. Doxycycline induction of dominant negative (DN)-PDX1 in transgenic INSrβ beta cells fully blocked hKLF11 function on hInsP underscoring the importance of the PDX1-p300 transcriptional complex for hKLF11 function in beta cells. We further observed loss of hKLF11 functions both in beta cells and in HEK293 cells after 5'-deletion or internal block mutation of the important PDX1 binding element A3. Of note, deletion or mutation of the other PDX1 binding sites within hInsP (A5, GG2 and A1) had no effect.

**Conclusion:** These results characterise hKLF11 as an important regulator of hInsP activity via the p300/PDX1 transactivation complex and the A3 cis acting element. Disturbance of these molecular mechanisms may contribute to the development of MODY7 in monogenic hKLF11 gene mutations.

## 508

**Expression of the Alx3 homeodomain gene in pancreatic beta cells is regulated by USF1/USF2 and glucose**

A. Fernandez-Perez, M. Mirasierra, P. Garcia-Sanz, M. Vallejo; Ciber de Diabetes y Enfermedades Metabólicas Ciberdem, Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, Spain.

**Background and aims:** Alx3 is an aristaless-type homeodomain transcription factor expressed in pancreatic islets. In previous studies we determined that Alx3 participates in the regulated expression of several islet genes, including insulin, glucagon, glucokinase and somatostatin. In addition, we demonstrated that lack of Alx3 in mice compromises islet cell survival leading to alterations in glucose homeostasis characterized by increased fasting blood glucose levels, impaired glucose tolerance, and age-dependent insulin resistance. Initial studies to investigate the mechanisms that regulate the expression of the Alx3 gene in pancreatic islets revealed the existence of multiple E-box promoter elements distributed over a region of approximately 2 kb of the promoter. In the present study, we sought to investigate the transcriptional mechanisms that regulate expression of Alx3 in pancreatic islets by identifying and analyzing functional promoter cis-DNA regulatory elements and the transcription factors that bind to them. In addition, we sought to determine whether expression of Alx3 in pancreatic islets is dependent on the concentration of glucose.

**Materials and methods:** Expression of Alx3 was assessed by quantitative RT-PCR. Luciferase reporter genes constructed with different regions of the mouse Alx3 promoter were transfected in islet- and non-islet-derived cell lines. PCR-based site directed mutagenesis of single or multiple promoter elements was used for functional studies of the promoter. Binding of nuclear

proteins to promoter elements was investigated by chromatin immunoprecipitation and electrophoretic mobility shift assays.

**Results:** Expression of Alx3 in MIN6 pancreatic beta cells was found to be proportionally dependent on the concentration of glucose. We identified 10 E-box type elements (named EB1–10) within a region of 2 kb of the Alx3 promoter. Mutation analyses followed by transfection in MIN6 indicated that at least four (EB1, EB4, EB8 and EB9/10) were found to be required for full transcriptional activity. Transcription factors USF1 and USF2 were found to bind preferentially to EB1 and EB8, respectively, by chromatin immunoprecipitation and electrophoretic mobility shift assays. In addition, both transcription factors were found to bind EB4 but not EB9/10. Transfections in insulin producing MIN6 cells using an expression vector encoding a dominant negative inhibitor of USF transcription factors indicated that they are required for full Alx3 promoter activity. We found that USF1 and 2 act synergistically, and that the integrity of at least EB1 and EB2 elements is required for these synergistic interactions. On EB1, USF1 appears to interact with the basal transcription machinery via direct contact with TFII-I. Finally, we found that Alx3 transcription is inducible by glucose, but this response is maintained in the presence of a dominant negative inhibitor of USF transcription factors.

**Conclusion:** Expression of Alx3 in pancreatic islet cells is regulated by the co-ordinated activity of USF1 and USF2 transcription factors binding concomitantly to at least three E-box promoter elements. USF transcription factors do not appear to be involved in glucose-regulated expression of Alx3.

Supported by: MINECO (BFU2011-24245 and CIBERDEM)

## 509

### Increase of beta cell mass via paracrine GLP-1 by selective non-viral GLP-1 gene therapy into alpha cells in intact pancreatic islets

J. Park<sup>1</sup>, H.-S. Jung<sup>2</sup>, J.-S. Lee<sup>2</sup>, N. Han<sup>1</sup>, S. Kim<sup>1</sup>, E. Lee<sup>1</sup>, T. Kim<sup>1</sup>, T. Kim<sup>1</sup>, M. Kwon<sup>1</sup>, S. Lee<sup>1</sup>, M. Park<sup>3</sup>, B. Rhee<sup>1</sup>, M. Kim<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, College of Medicine, Inje University,

<sup>2</sup>Paik Institute for Clinical Research, Inje University, <sup>3</sup>Department of Internal Medicine, College of Medicine, Dong-A University, Busan, Republic of Korea.

**Background and aims:** Glucagon-like peptide-1 (GLP-1) is an incretin hormone expecting improvements in beta-cell survival and increase in beta cell mass. To overcome the short half-life of GLP-1, several groups have explored GLP-1 gene therapy in pancreatic beta cells. But, we have known that alpha cells were increased and beta cells were decreased with progression of diabetes. Long term safety of viral gene therapy is still major concern and beta cell proliferative effect is not clear. We rationalized that it may be desirable to have local GLP-1 production within the islet without raising peripheral levels. Therefore, we try to induce transient high concentration of GLP-1 expression in pancreatic alpha cells by sonoporation and find out their effects on proliferation of adjacent pancreatic beta cells

**Materials and methods:** Alpha TC-1 & Beta TC-6 cell was co-cultured with D-MEM medium and islets were isolated from wistar rats and maintained with M199 medium. Co-cultured alpha/beta cells or islet applied the mixture of pGlu-GLP-1 with PBS and then performed sonoporation (frequency, 1.0 MHz; duty, 20 %; intensity, 2.0 W/cm<sup>2</sup>; time, 30 s) at plate bottom using Sonitron 2000 ultrasonicator with a probe of diameter 6 mm. After 24hr or 48hr transfection, cells or islets were stained with ki-67 to confirm beta-cell proliferation change.

**Results:** Expression of GLP-1 mRNA and protein were detected in transfected alpha-TC-1 cell. Colocalization of GLP-1 and glucagon immunoreactivity was shown within the alpha-cells of islets. The levels of GLP-1 secreted from a-TC cells were over 10nmol. In co-culture with alpha and beta- cells, beta-TC-6 cell mass was increased. Ki-67 in beta cell of islet was significantly increased with transfected alpha cells.

**Conclusion:** Alpha cell targeted GLP-1 gene therapy by sonoporation led to localized GLP-1 expression in alpha cells and proliferation of beta cells. It will be another strategy to overcome.

## 510

### The role of Rb and its family protein p107 in regulating cell cycle of pancreatic beta and alpha cells

E.P. Cai<sup>1,2</sup>, X. Wu<sup>3</sup>, S.A. Schroer<sup>2</sup>, E. Zacksenhaus<sup>2</sup>, M. Woo<sup>2,4</sup>;

<sup>1</sup>Institute of Medical Science, University of Toronto, Canada, <sup>2</sup>Toronto

General Research Institute, University of Toronto, Canada, <sup>3</sup>The First

Affiliated Hospital, Nanjing Medical University, China, <sup>4</sup>Department of Medicine, St. Michael's Hospital, Toronto, Canada.

**Background and aims:** Insulin insufficiency and glucagon excess are considered to be major contributors for diabetes development. Pancreatic  $\beta$ -cells can re-enter cell cycle to compensate for increased insulin demand. Moreover, insulin deficiency due to  $\beta$ -cell death can stimulate  $\alpha$ -cell expansion and hyperglucanemia, further contributing to hyperglycemia. Therefore, evaluation of cell cycle regulation in both  $\beta$ - and  $\alpha$ -cells is essential for better understanding diabetes pathogenesis. Retinoblastoma protein (Rb) and its related protein, p107, play a critical role in cell cycle arrest and tissue differentiation but appear to be dispensable for post-mitotic cells. Rb plays a minor role in well-differentiated  $\beta$ -cells as shown in rat insulin promoter-driven Rb knockout islets. However, the effects of Rb in proliferating  $\beta$ - and  $\alpha$ -cells remain elusive.

**Materials and methods:** To investigate the role of Rb in proliferating  $\beta$ - and  $\alpha$ -cells, we have deleted Rb via Pdx1-Cre, and Pdx1-Cre:Rb<sup>fl/fl</sup> will be referred herein as RbKO mice. To assess the role of its family member p107, we used whole-body p107 knockout mice (p107KO), and Rb/p107 double knockout (DKO) mice were generated to assess the combined roles of these Rb family members in proliferating pancreatic  $\beta$ - and  $\alpha$ -cells.

**Results:** RbKO mice demonstrated improved glucose tolerance ( $p < 0.05$ ) without changes in insulin sensitivity, while p107KO had similar glucose tolerance compared to their littermate wildtype (WT) controls. Pancreatic sections of RbKO mice demonstrated increased  $\beta$ -cell area (+35%,  $p < 0.05$ ) and their islets exhibited enhanced  $\beta$ -cell function as assessed by in vivo glucose-stimulated insulin secretion ( $p < 0.05$ ) and increased protein levels of p-IRS1/2 (2.1-fold,  $p < 0.01$ ), p-Akt (2.0-fold,  $p < 0.01$ ), Pdx1 (2.9-fold,  $p < 0.001$ ), CyclinD1 (1.6-fold,  $p < 0.01$ ) and E2F1 (1.7-fold,  $p < 0.001$ ) but decreased p53 (0.7-fold,  $p < 0.01$ ), p27 (0.6-fold,  $p < 0.001$ ) and p21 (0.7-fold,  $p < 0.01$ ) levels. DKO mice showed improved glucose tolerance ( $p < 0.01$ ); however, this was abolished with aging. Both RbKO and DKO mice showed increased  $\beta$ -cell proliferation as assessed by Ki67 staining at 8 wks (2.2-fold,  $p < 0.05$ ; 1.7-fold,  $p < 0.05$ ). However, with aging, there was also a concomitant increase in  $\beta$ -cell apoptosis in DKO mice (2.5-fold,  $p < 0.05$ ), resulting in a net loss of  $\beta$ -cell mass in older (18–22 wks) DKO mice. Interestingly, RbKO and DKO mice demonstrated reduced glucagon levels compared to WT controls (74.1 $\pm$ 4.6 pg/ml in WT, 40.8 $\pm$ 3.3 pg/ml in RbKO, 46.1 $\pm$ 5.4 pg/ml in DKO;  $p < 0.001$ ) and  $\alpha$ -cell area (-60%,  $p < 0.001$ ; -93%,  $p < 0.001$ ), and reduced  $\alpha$ -cell developmental gene expressions, *arx* (-80%,  $p < 0.01$ ; -93%,  $p < 0.01$ ) and *pax6* (-63%,  $p < 0.001$ ; -66%,  $p < 0.001$ ).

**Conclusion:** These data reveal that Rb may display a dichotomous role in  $\beta$ - and  $\alpha$ -cells, whereby Rb loss leads to an increase in  $\beta$ -cell mass and a concomitant decrease in  $\alpha$ -cells. Additionally, combined loss of Rb and p107 leads to an age-dependent depletion of  $\beta$ -cells by apoptosis. Further understanding of the complexity in regulation of cell cycle, viability and differentiation of  $\beta$ - and  $\alpha$ -cells will provide new strategies for treatment of diabetes.

Supported by: CDA DSRA, CDA Grant-in-Aid, CIHR MOP-201188 and MOP-191501



## PS 029 Beta cell lipotoxicity

511

### RNA-sequencing identifies dysregulation of the human pancreatic islet transcriptome by the saturated fatty acid palmitate

M. Cnop<sup>1</sup>, G. Bottu<sup>1</sup>, T. Griebel<sup>2</sup>, B. Abdulkarim<sup>1</sup>, L. Marselli<sup>3</sup>, P. Marchetti<sup>3</sup>, M.I. McCarthy<sup>4</sup>, M. Sammeth<sup>2</sup>, D.L. Eizirik<sup>1</sup>;

<sup>1</sup>Laboratory of Experimental Medicine, Université Libre de Bruxelles, Brussels, Belgium, <sup>2</sup>Functional Bioinformatics, Centre Nacional d'Anàlisi Genòmica, Barcelona, Spain, <sup>3</sup>Department of Endocrinology and Metabolism, University of Pisa, Italy, <sup>4</sup>Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK.

**Background and aims:** Pancreatic beta cell dysfunction and death are central in the pathogenesis of type 2 diabetes but the underlying mechanisms are not fully understood. Saturated free fatty acids may cause beta cell failure and contribute to diabetes development in genetically predisposed individuals.

**Materials and methods:** RNA-sequencing was used to identify transcripts expressed in five human islet preparations, basally or following a 48-hour exposure to the saturated fatty acid palmitate (0.5 mM in the presence of 1% albumin). Organ donors were aged 55±9 years. Beta cell purity of the islets assessed by insulin immunostaining was 50±5%. Samples were sequenced on Illumina Genome Analyzer II and data analyzed using GEM mapper and Flux Capacitor software. Transcript expression was considered changed by Benjamini-Hochberg-corrected Fisher testing and if it changed significantly in ≥4/5 samples and in the opposite direction in none. Genes were annotated manually or using Ingenuity Pathway Analysis or DAVID.

**Results:** A total of 30,026 transcripts corresponding to 19,882 genes were expressed in the human islets. Palmitate induced 428 genes and downregulated 897 genes ( $p < 0.05$ ). These included genes regulating the endoplasmic reticulum stress response, ubiquitin and proteasome function, autophagy and apoptosis. Transcripts related to innate immunity were upregulated and several HLA transcripts were downregulated. Potassium channels and transcription factors controlling beta cell phenotype were inhibited by palmitate. Confirmation and functional studies of key genes are ongoing. 52/63 of the candidate genes for type 2 diabetes were expressed in human islets with an RPKM >1, and palmitate modified expression of 11 of these. Palmitate caused a shift in alternative splicing in 574 transcripts in human islets. Ingenuity Pathway Analysis of modified transcripts and genes confirmed that top changed functions related to cell death and cellular development. DAVID analysis of transcription binding sites in palmitate-modified transcripts revealed a role for the endoplasmic reticulum stress response regulators XBP-1 and ATF6, among others.

**Conclusion:** Using RNA-sequencing we mapped the human islet transcriptome and identified novel mechanisms of palmitate-induced beta cell dysfunction and death. These data also point to crosstalk between metabolic stress and several type 2 diabetes candidate genes at the beta cell level.

*Supported by: FP7 CEED3, FP7 BetaBat, JDRFI*

512

### FABP5 depletion protects from STZ induced diabetes

K. Zien<sup>1</sup>, J. Bergemann<sup>1</sup>, A. Meyer<sup>1</sup>, Y. Owada<sup>2</sup>, K. Maedler<sup>1</sup>;

<sup>1</sup>Centre for Biomolecular Interactions, University of Bremen, Germany,

<sup>2</sup>Department of Organ Anatomy, Minamikogushi, Japan.

**Background and aims:** Fatty acid binding proteins (FABPs) facilitate the uptake, transport and metabolism of fatty acids within the aquatic environment of the cell. FABP5 (E-FABP, mal1) polymorphisms are reported to be associated with type 2 diabetes in humans and a depletion of FABP5 in mice results in a protection from insulin resistance and diabetes upon high fat diet. FABP5 is expressed in rat islets and in the  $\beta$ -cell line INS1E of rat origin. Subsequently, we asked the question if FABP5 is also expressed in human and mouse islets and whether it regulates  $\beta$ -cell survival and function.

**Materials and methods:** FABP5<sup>-/-</sup> and wildtype mice were injected with a single dose streptozotocin (STZ, 100 mg/kg body weight) followed by an 8-week feeding of high fat/ high sucrose diet (HFD). Intraperitoneal glucose tolerance (IpGTT) and insulin tolerance tests (IpITT) were performed before and after STZ as well as during the diet.  $\beta$ -cell mass was analyzed by microscopical scanning of the tissue,  $\beta$ -cell survival and proliferation was monitored using double-staining for TUNEL or Ki67 and insulin. Isolated islets of wildtype and FABP5<sup>-/-</sup> mice were treated with a combination of glucose

(22.2mM) and palmitate (500 $\mu$ M) for three days and  $\beta$ -cell survival and function were monitored.

**Results:** FABP5 was expressed in human and mouse islets. STZ treatment of wildtype mice resulted in an 2.5-fold increase in fed blood glucose within two weeks while blood glucose increased only to 1.6-fold in FABP5<sup>-/-</sup> mice. WT mice injected with STZ showed impaired glucose tolerance after 2 weeks while this was partially prevented in FABP5<sup>-/-</sup> mice. After 8 weeks on HFD, STZ treated wildtype mice were completely glucose intolerant, while FABP5<sup>-/-</sup> mice showed a glucose tolerance similar to non-STZ-treated wildtype and FABP5<sup>-/-</sup> controls. The  $\beta$ -cell mass of wildtype mice was 50% reduced upon STZ-HFD treatment, while FABP5<sup>-/-</sup> mice had a 1.6-fold increased  $\beta$ -cell mass at all conditions, compared to wildtype mice. To further investigate lipotoxic effects on islets, we exposed isolated islets from FABP5<sup>-/-</sup> and wildtype mice to a pro-diabetic milieu. While the combination of glucose and palmitate induced apoptosis by 4-fold, islets from FABP5<sup>-/-</sup> mice were not affected. No change in proliferation could be observed.

**Conclusion:** Our data show, that the depletion of FABP5 improves  $\beta$ -cell function, survival and mass.

*Supported by: DFG*

## PS 029 Beta cell lipotoxicity

513

### The p66Shc isoform mediates FFA-induced apoptosis in pancreatic beta cells

A. Natalicchio<sup>1</sup>, F. Tortosa<sup>1</sup>, R. Labarbuta<sup>1</sup>, G. Biondi<sup>1</sup>, M.R. Orlando<sup>1</sup>, A. Leonardini<sup>1</sup>, A. Cignarelli<sup>1</sup>, R. Ficarella<sup>1</sup>, E. Carchia<sup>2</sup>, P. Marchetti<sup>3</sup>, S. Perrini<sup>1</sup>, L. Laviola<sup>1</sup>, F. Giorgino<sup>1</sup>;

<sup>1</sup>Endocrinology & Metabolic Diseases, University of Bari, <sup>2</sup>Biogem, Ariano Irpino (Avellino), <sup>3</sup>Endocrinology and Metabolism of Transplantation, AOU, University of Pisa, Italy.

**Background and aims:** The p66Shc protein, through its phosphorylation on Ser36, regulates oxidative stress and cell survival in multiple cell types. Saturated FFA, if chronically in excess, can reduce insulin biosynthesis and secretion and induce beta-cell apoptosis, a phenomenon that has been termed “lipotoxicity”. In this study, the role of p66Shc in the FFA-dependent beta-cell apoptosis was explored.

**Materials and methods:** Expression and phosphorylation levels of specific signaling molecules were assessed by immunoblotting techniques. Gene expression was evaluated by real-time RT-PCR. Beta-cell apoptosis was quantified by an ELISA assay evaluating oligosome release into the cytosol and by detection of cleaved caspase-3 protein levels. Wild-type p66Shc and mutant p66Shc, in which Ser36 was replaced by Ala (p66Shc-Ala36), were selectively overexpressed by infecting cells with recombinant adenoviruses. Silencing of p66Shc was obtained following beta-cell transfection with two independent siRNAs.

**Results:** Exposure of rat INS-1E insulinoma cells and both human and mouse islets to 0.5 mM palmitate induced a selective 2- to 3-fold increase in both mRNA and protein levels of p66Shc ( $p<0.05$ ), respectively, without affecting the other Shc protein isoforms; furthermore, palmitate enhanced p66Shc phosphorylation on Ser36 ( $p<0.05$ ). When INS-1E cells were pre-treated with pifithrin- $\alpha$ , an inhibitor of the p53 protein, the palmitate-induced increase in p66Shc expression was completely abrogated ( $p<0.05$ ). Adenovirus-mediated overexpression of p66Shc resulted in increased phosphorylation of p66Shc on Ser36 and beta-cell apoptosis compared to control, both under basal conditions and following exposure to palmitate ( $p<0.05$ ). By contrast, overexpression of a phosphorylation-defective p66Shc protein, with Ser36 mutation to Ala, did not affect basal and reduced palmitate-induced apoptosis ( $p<0.05$ ). In addition, palmitate increased JNK phosphorylation, and cells with p66Shc overexpression showed increased basal and palmitate-induced JNK phosphorylation compared with control cells ( $p<0.05$ ). Inhibition of JNK phosphorylation by SP600125 prevented basal and palmitate-induced phosphorylation of p66Shc on Ser36 and beta-cell apoptosis ( $p<0.05$ ). Moreover, when the palmitate-dependent increase of p66Shc was inhibited using pifithrin- $\alpha$  or a specific p66Shc siRNA, palmitate-induced apoptosis was abrogated ( $p<0.05$ ). Finally, in pancreatic islets from cadaveric donors, p66Shc mRNA levels were found to be increased in obese/overweight (BMI>25) compared with lean (BMI<25) subjects ( $p<0.05$ ).

**Conclusion:** In pancreatic beta-cells the FFA palmitate triggers the p53-mediated upregulation of the p66Shc protein, which is phosphorylated on Ser36 by JNK, and mediates a pro-apoptotic response. Thus, p66Shc acts as a novel signaling intermediate in the FFA-mediated beta-cell damage, and is up-regulated in human obesity. p66Shc may thus represent a potential novel target to prevent the deleterious effects of lipotoxicity on pancreatic beta-cells.

514

### Unsaturated fatty acids protect against reactive oxygen species mediated lipotoxicity in insulin-producing cells

M. Elsner<sup>1</sup>, W. Gehrmann<sup>1</sup>, A. Jörns<sup>1,2</sup>, S. Lenzen<sup>1</sup>;

<sup>1</sup>Institute of Clinical Biochemistry, <sup>2</sup>Centre of Anatomy, Hannover Medical School, Germany.

**Background and aims:** Elevated levels of non-esterified fatty acids (NEFAs) can cause beta cell dysfunction and apoptosis in T2DM. Palmitic acid, the physiologically most abundant saturated NEFA, has a strong cytotoxic effect upon insulin-producing cells, whereas unsaturated NEFAs are not toxic and can even protect against the toxicity of long chain saturated NEFA induced lipotoxicity. Long chain NEFAs can be metabolized not only in the mitochondria but also in the peroxisomes. In contrast to mitochondrial  $\beta$ -oxidation, the acyl-CoA oxidases in the peroxisomes generate hydrogen peroxide

( $H_2O_2$ ) and not reducing equivalents. Since  $\beta$ -cells lack almost completely catalase expression they are exceptionally vulnerable by  $H_2O_2$ . It was the aim of the present study to analyse the structural requirement of unsaturated NEFAs for the protection against saturated NEFA mediated toxicity in insulin producing cells and to investigate the mechanism of protection.

**Materials and methods:** Cell viability of insulin-producing RINm5F cells was determined by MTT assay after 24 h exposure to palmitic acid (PA) and different unsaturated NEFAs. The induction of apoptosis was analysed by caspase-3 assay. DCF was used to quantify reactive oxygen species formation in RINm5F cells overexpressing catalase after exposure to PA and oleic acid (OA). For specific cellular analysis of hydrogen peroxide generation after incubation with PA and OA the  $H_2O_2$  sensitive protein HyPer was overexpressed in the peroxisomes of RINm5F cells and primary rat beta cells. Ultrastructural changes in the morphology of RINm5F cells caused by NEFAs were analysed by electron microscopy.

**Results:** The toxicity of saturated NEFAs and the protective effect of unsaturated NEFAs decreased with shortening of the chain length. The  $EC_{50}$  value for palmitic acid (C16:0) was around 100  $\mu$ M. While oleic (C18:1), linoleic (18:2), gamma linoleic acid (18:3) and palmitoleic acid (C16:1) had a similar strong protective effect, myristoleic acid (C14:1) and dodecenoic acid (C12:1) were less effective against PA induced toxicity. The induction of caspase-3 by PA was inhibited through incubation with OA. Only overexpression of catalase in the peroxisome or a co-incubation with OA reduced PA induced ROS generation to the same level of untreated control cells. In primary rat beta cells overexpressing the hydrogen peroxide sensor protein HyPer the  $H_2O_2$  production increased 10fold after PA incubation in comparison to untreated cells while OA treatment results not in a significant increase in  $H_2O_2$  production. Moreover OA was able to prevent the PA mediated induction of hydrogen peroxide production. The EM analyses revealed after incubation with PA clear damage of the rough ER, while the mitochondria remained intact. The cisternae of the ER were elongated and dilated containing dark deposits. When the cells were incubated with OA lipid droplets accumulated in the cytoplasm, whereas the ER remained unaffected. Minor accumulation of lipid droplets and damage to the ER were observed when the cells were treated with a mixture of PA and OA.

**Conclusion:** The protective effect of unsaturated NEFA against lipotoxicity was chain-length dependent but independent on the number of double bonds. OA was able to prevent PA induced  $H_2O_2$  production in insulin producing RINm5F cells and primary rat beta cells. Our findings indicate that ROS play an important role for lipotoxicity.

*Supported by: European Union (Integrated Project EuroDia LSHM-CT-2006-518153)*

515

### Evidence of reduced ER-to-Golgi trafficking of endogenous cargo protein in lipotoxic beta cells and islets of db/db mice

N. Sue, J.Y. Chan, D.R. Laybutt, T.J. Biden;

Diabetes and Obesity Program, Garvan Institute of Medical Research, Darlinghurst, Australia.

**Background and aims:** Saturated fatty acids lead to a trafficking defect in pancreatic beta cells, which contributes to ER stress via protein overload. A VSVG reporter protein has been used previously to demonstrate delayed ER-to-Golgi trafficking in the presence of palmitate. Our aim was to confirm this defect for endogenous cargo in the beta cell, and to extend the characterisation further by investigating whether there was a selectivity of this trafficking delay for particular cargo proteins, or if it was a generalised phenomenon. We also wanted to explore this ER-to-Golgi trafficking defect in the context of an animal model of diabetes. To this end, we monitored changes in the expression level of a number of ER-Golgi secretory pathway related genes in islets of *db/db* mice as they progressed from a pre-diabetic to diabetic state.

**Materials and methods:** Vesicle budding assays were used to reconstitute the process of ER-to-Golgi trafficking in a cell free system. In this assay, bulk cytosol extracted from mouse liver was incubated at 37°C with a microsomal (chiefly ER) fraction isolated from MIN6  $\beta$ -cells pre-treated for 48 hours with palmitate (0.4 mmol/l Palm: 0.92% BSA) or control BSA. Secretory vesicles bud off from the ER membranes, and are separated from membranes by differential centrifugation. The incorporation of endogenous cargo proteins into the budded off vesicles was monitored by immunoblotting. Islets were isolated from *db/db* and control mice at 6 (pre-diabetic) and 16 (diabetic) weeks of age. Gene expression of a number of secretory pathway genes was assessed by quantitative PCR.

**Results:** Vesicle budding assays revealed that there was a time-dependent increase in vesicular trafficking of the endogenous cargo protein Carboxypeptidase E (CPE). This budding was diminished in microsomes from palmitate pre-treated MIN6 cells (fold enrichment in vesicles: Control  $4.48 \pm 2.3$ ; palmitate  $2.48 \pm 0.3$ ;  $n=2$ ), even after allowing for the reduced CPE protein levels in response to palmitate treatment. We also studied *db/db* mice which are a model of beta cell dysfunction and type 2 diabetes. In their islets, the expression of a variety of secretory pathway genes was down-regulated in the progression to the diabetic state. These include significant reductions in Sec24a (52%), Sec24d (35%), Lman1 (53%), Rpn1 (40%), and Ssr3 (31%) ( $p<0.05$  *db/db* 6 week vs *db/db* 16 week by t-test,  $n=8-12$ ). Conversely, the expression levels of these genes did not change in the control mice over the same period. These reductions are consistent with a down-regulation of ER-to-Golgi trafficking.

**Conclusion:** We have employed two independent approaches to provide support to the concept that protein trafficking through the early secretory pathway in pancreatic beta cells is adversely affected by lipid oversupply and in the context of diabetes.

Supported by: NHMRC of Australia

## 516

### HDLs use multiple pathways to protect pancreatic beta cells against ER stressors

C. Widmann<sup>1</sup>, J. Puyal<sup>2</sup>, G. Dubuis<sup>1</sup>, J. Pétremand<sup>1</sup>;

<sup>1</sup>Physiology, <sup>2</sup>Cell Biology and Morphology, University of Lausanne, Switzerland.

**Background and aims:** HDLs protect pancreatic beta cells against apoptosis induced by several ER stressors, including thapsigargin, cyclopiazonic acid, palmitate and insulin over-expression. This protection is mediated by the capacity of HDLs to maintain proper ER morphology and ER functions such as protein folding and trafficking. Here we investigated whether HDLs used a similar pathway to protect beta cells against tunicamycin (TM), a protein glycosylation inhibitor inducing ER stress.

**Materials and methods:** The Min6 insulinoma cell line was incubated with TM in the presence or in the absence of HDLs. The extent of apoptosis was determined by scoring the number of cells displaying pyknotic nucleus. ER stress was assessed by measuring the induction of various stress markers (XBP1 splicing, CHOP and BiP induction, PERK activation, etc). Protein folding and trafficking from the ER to the Golgi was investigated using a temperature-sensitive mutant of a vesicular stomatitis virus glycoprotein (VSVG) fused to GFP whose folding and trafficking can be monitored. ER morphology was assessed by electron microscopy.

**Results:** HDLs inhibited apoptosis induced by tunicamycin in Min6 cells. Surprisingly, this protection was neither associated with a reduction in ER stress nor with restoration of protein folding and trafficking in the ER.

**Conclusion:** These data indicate that HDLs can use at least two mechanisms to protect beta cells against ER stressors. One that relies on the maintenance of ER function and one that operates independently of ER function modulation. The capacity of HDLs to activate several anti-apoptotic pathways in beta cells may explain their ability to efficiently protect these cells against a variety of insults.

## 517

### Subtle alterations in sphingolipid and cholesterol content within the endoplasmic reticulum (ER) of pancreatic beta cells underlies ER dysfunction, stress and lipoapoptosis

E. Boslem<sup>1</sup>, G. MacIntosh<sup>2</sup>, P.J. Meikle<sup>2</sup>, T.J. Biden<sup>1</sup>;

<sup>1</sup>Diabetes and Obesity, Garvan Institute of Medical Research, Sydney, <sup>2</sup>Baker IDI Heart and Diabetes Institute, Melbourne, Australia.

**Background and aims:** Fatty acid (FA) oversupply contributes to the  $\beta$ -cell death that occurs in type 2 diabetes. This death is mediated in part by chronic, unresolved endoplasmic reticulum (ER) stress signalling and is specific to saturated FA exposure. Since we have previously implicated sphingolipid metabolism and defective protein trafficking in this process, our current aim was to identify the exact metabolite and mechanism of action underlying this cytotoxicity.

**Materials and methods:** MIN6 cells, were treated chronically (48 h) with 0.4mM palmitate (palm):0.92% BSA, as a mild model of lipid oversupply. The level of apoptosis (DNA fragmentation ELISA) was determined fol-

lowing palm treatment and in combination with genetic intervention via overexpression (oe). Other endpoint analyses included lipidomics via mass spectrometry, western blotting of ER stress proteins and measurement of cholesterol regulated genes via a luciferase tagged sterol regulatory element (SRE) reporter.

**Results:** Palm treatment caused an overall decrease in ER sphingomyelin (SM) content (-38%,  $p=0.06$ ) as well as a localized increase in ER ceramide (Cer) content (56%,  $p=0.04$ ). Consistent with this was a 0.67 fold decrease ( $p=0.06$ ) in mRNA expression levels of ceramide transport protein (CERT) responsible for ER-to-golgi transport of Cer. Unsaturated FA oleate, did not suppress transcription of CERT and augmented its activation. Inhibiting CERT function was also sufficient to augment ER stress in the presence of palm (2 fold). However, oe of wild type or a constitutively active CERT mutant did not protect from lipoapoptosis or ER stress, which argued against ER Cer as playing a causative role in either defect. The inability of glucosylceramide synthase (GCS) oe to prevent ER Cer accrual, a strategy previously shown to protect from lipoapoptosis, ER stress and the palm-induced vesicular traffic delay, agreed with this conclusion. The palm-induced decrease in ER SM content was hence investigated, and a concomitant decrease in free cholesterol (FC) at the ER (-28%) and plasma membrane (-20%) was found, which stimulated SRE-driven gene expression (2.2 fold). These reductions were reversed by GC synthase (GCS) oe. Reciprocal modulations of apoptosis occur when the proportion of Cer to SM is altered via SM synthase 1 oe (-25%) or neutral ER SMase oe (20%) consistent with a regulatory role for ER SM content. SM and FC are associated in lipid rafts therefore perturbations of these ER microdomains may disturb  $\beta$ -cell subcellular membrane function. This might also explain alterations in downstream proinsulin to insulin conversion (proinsulin/insulin -43% with palm, +170% palm+SMS1 oe).

**Conclusion:** Subtle alterations in SM and FC content of the ER potentially underlie this organelle's dysfunction that leads to ER stress and lipoapoptosis. This may impact on vesicle membrane dynamic processes such as budding and golgi cisternal progression. This may be the source of the protein backlog previously hypothesised to cause the ER-to-golgi protein vesicular trafficking delay. Enhanced activation of CERT by oleate may be important for its cytoprotective action of unsaturated FAs.

Supported by: NHMRC Project Grant

## 518

### GRP78 overexpression in pancreatic beta cells protects from high fat diet-induced diabetes in mice

A. Volchuk<sup>1</sup>, T. Teodoro<sup>2</sup>, I. Schuiki<sup>1</sup>, L. Zhang<sup>1</sup>;

<sup>1</sup>Cellular & Molecular Biology, Toronto General Research Institute,

<sup>2</sup>Biochemistry, University of Toronto, Canada.

**Background and aims:** Endoplasmic reticulum (ER) stress is a potential mechanism involved in type 2 diabetes pathology and has been shown to contribute to both insulin resistance in liver and adipose tissue and pancreatic beta-cell dysfunction. We hypothesized that increased ER chaperone capacity would protect pancreatic beta-cells from dysfunction resulting from obesity and improve glucose homeostasis in mice on a chronic high fat diet (HFD).

**Materials and methods:** To examine whether ER stress in pancreatic beta-cells contributes to the development of type 2 diabetes we generated a mouse model that over-expresses the resident ER chaperone and unfolded protein response regulator GRP78/BiP in pancreatic beta-cells under the control of a rat insulin promoter. Transgenic and control mice were challenged with a HFD and metabolic parameters and ER stress markers in islets were analyzed.

**Results:** In transgenic C57B/6 mice expression of the RIP-GRP78myc transgene was restricted to pancreatic beta-cells in islets and was approximately 8-fold higher compared to transgene negative control mice. Transgenic mice had normal weight gain, metabolic physiology and islet architecture on a regular chow diet. Control mice on a 20-week HFD (45% kcal from fat) developed obesity, glucose intolerance, insulin resistance and islet hypertrophy. Remarkably, GRP78 transgenic mice tended to be leaner than their transgene negative littermates and were protected from glucose intolerance and insulin resistance. Furthermore, islets from transgenic mice had reduced hypertrophy and the mice were less hyperinsulinemic compared to controls on the HFD. In control animals HFD induced mild ER stress in islets, which correlated with reduced islet GLUT2 expression. Islets from transgenic mice were protected from ER stress and had increased cell surface GLUT2 expression compared to control mice on the HFD.

**Conclusion:** Overall, these data show that increased chaperone capacity in pancreatic beta-cells protects from ER stress and the pathogenesis of obesity-



induced type 2 diabetes. By maintaining pancreatic beta-cell function whole body glucose homeostasis is improved in transgenic mice on a HFD.

Supported by: Canadian Institutes for Health Research (CIHR)

## 519

### A proteomic investigation of the response induced by palmitate and thapsigargin in the insulin producing cell line INS-1E

V. Rosengren<sup>1</sup>, H. Johansson<sup>2</sup>, J. Lehtiö<sup>2</sup>, Å. Sjöholm<sup>1</sup>, H. Orsäter<sup>1</sup>;

<sup>1</sup>Department of Clinical Science and Education, Södersjukhuset,

<sup>2</sup>Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** Pancreatic beta-cells have a highly developed endoplasmic reticulum (ER) in order to meet the high demand for synthesis of insulin. Saturated fatty acids has been shown to induce the ER stress response in pancreatic beta-cells and this has been put forwarded as a mechanism for fatty acid toxicity in these cells. The aim of this study was to compare the cellular protein changes in INS-1E cells after exposure to the saturated fatty acid palmitate (Palm) and the ER stress inducer thapsigargin (Th), to investigate the regulation of proteins inside ER and the connection to other parts of the cell.

**Materials and methods:** INS-1E cells were treated with 0.4mM Palm or 200nM Th for 0, 4, 16 or 24 hours (h). After treatment cellular protein were isolated and subjected to iTRAQ labelling using 8 different isobaric tags for each data set. Analysis of the peptides was performed by LC/MS/MS. Identification was performed against the Uniprot database. ProteinCenter was used to categorize proteins according to GO annotations and Ingenuity Pathway Analysis (IPA) was used for investigation of canonical pathways and networks. Induction of apoptosis were investigated by measuring DNA fragmentation after treatment.

**Results:** Used concentrations of Th and Palm results in the same degree of apoptosis after 24 h of treatment. Proteomic analysis with iTRAQ offers a way to identify and quantify 8 samples in one assay. Two independent samples from each time point were combined in the analysis resulting in Two data sets. From the identification of 7073 unique proteins, 1% FDR, 5673 proteins were overlapping between the data sets and used for further analysis. Differentially expressed proteins were extracted and resulted in 1236 proteins after Th treatment and 272 proteins after Palm. Investigation of the regulated proteins in ProteinCenter reveals that 4 h of treatment with Th leads to over represented ER proteins while ribonucleoprotein complexes are under represented. At the time points 16 and 24 h ribonucleoprotein complexes are still under represented as well as the biological process RNA processing. Treatment with Palm for 16 h leads to over represented spindle proteins while the over represented biological processes are M-phase and lipid metabolic process. Investigation of regulated proteins in IPA reveals that networks involved in cell cycle and cell death were over represented after Th treatment. Palm showed over representation of networks involved in lipid metabolism, small molecule biochemistry and molecular transport. Pathways over represented after Th treatment were Biosynthesis of steroids, Polo-like kinases and protein ubiquitination pathway. Pathways over represented after Palm treatment were Polo-like kinases, fatty acid elongation in mitochondria and fatty acid biosynthesis.

**Conclusion:** The results from this study provides a large number of identified proteins, 5673 unique proteins positively identified in two independent data sets. Experiments with time series data can give valuable information about the dynamic regulation of proteins since activated processes can vary over time. Although both Palm and Thaps induced the same extent of apoptosis in these experiments the qualitative effects on the proteome was different. The treatment intervened with cellular RNA handling which was an effect not shared by Palm.

## 520

### Impaired islet expression of islet brain 1 in diabetes and in response to chronic palmitate exposure: role for proteasome and inducible cAMP early repressor

S. Brajkovic<sup>1</sup>, F. Wetter<sup>1</sup>, B. Lefebvre<sup>2</sup>, F. Pattou<sup>2</sup>, G. Waeber<sup>3</sup>, A. Abderrahmani<sup>4</sup>;

<sup>1</sup>Department of Cell Biology and Morphology, Lausanne, Switzerland,

<sup>2</sup>University of Lille Nord de France, Department of Endocrine Surgery-

Lille University Hospital, France, <sup>3</sup>Service of Internal Medicine, Lausanne, Switzerland, <sup>4</sup>University of Lille Nord de France, France.

**Background and aims:** The mitogen activated protein kinase 8 interacting protein 1 also called JNK interacting protein 1 or islet brain 1 (IB1) is candidate gene for T2D. IB1 maintains appropriate insulin expression and  $\beta$ -cells survival against pro-apoptotic stimuli. Decrease in the IB1 contents is thought to account for  $\beta$ -cells death evoked by most diabetogenic environment, and is deemed to contribute to the development of diabetes. Chronic exposure of islet  $\beta$ -cells to high concentrations of the saturated free fatty acid palmitate exerts adverse effects on  $\beta$ -cells tasks and cells survival. The aim of the study was to determine whether reduction of IB1 expression contributes to the deleterious effects of palmitate in beta cells.

**Material and methods:** Human islets were isolated from brain-dead organ donors with type 2 diabetes or not. Palmitate was coupled to bovine serum albumin in a molar ratio of 5:1. Human isolated islets, as well as mouse insulin secreting MIN6 cells were cultured with palmitate at different incubation times and concentrations. The protein and mRNA contents were analyzed by Western blotting experiments and quantitative real-time PCR, respectively.

**Results:** Prolonged incubation of insulin-producing cells to palmitate 0.5 mM decreased the IB1 protein contents at low and high glucose concentration. Loss of IB1 proteins appeared at 24 hours incubation. The ER stress inducers such as thapsigargin mimicked the effects of palmitate on the IB1 abundance. Reduction in the IB1 levels resulted from increased proteasome-mediated degradation but was independent of unfolded protein response. Chemical chaperones were unable to prevent reduction of IB1 caused by palmitate or thapsigargin, whereas proteasome inhibitors permitted a full restoration in the IB1 contents. Beside of the protein contents, chronic palmitate elicited a decrease in the IB1 mRNA levels. This diminution was observed in isolated human islets incubated with palmitate and in diabetic islets. Reduction of the IB1 transcripts caused by palmitate was associated with an increase in the inducible cAMP early repressor (ICER), a transcription factor known to repress IB1 in  $\beta$ -cells.

**Conclusion:** Palmitate reduces the levels of the cyto-protective IB1 by promoting protein degradation and by affecting transcriptional activity through ICER. This mechanism could account for islets dysfunction in diabetes.

Supported by: the SNF, Chair of Excellence ANR N°ANR-10-CEXC-0

## PS 030 Beta cell glucotoxicity and oxidative stress

521

### Amino acid toxicity in pancreatic beta cells, a potential new link between obesity and type 2 diabetes

N. Mullooly<sup>1</sup>, D. Smith<sup>2</sup>, P. Newsholme<sup>1,3</sup>;<sup>1</sup>University College Dublin, Co. Roscommon, Ireland, <sup>2</sup>Astrazeneca, Manchester, UK, <sup>3</sup>Curtin University, Perth, Australia.

**Background and aims:** Obesity and the metabolic syndrome are associated with excessive lipid and protein intake. Recent metabolic profiling studies have demonstrated strong correlations between branched-chain amino acid levels and type 2 diabetes susceptibility. Glucose and lipids in chronic excess have toxic effects on pancreatic beta cells but the effect of hyperaminoemia on beta cell function has never been investigated to date. The aim of this study was to investigate the effect of prolonged elevations in amino acid levels on beta cell function in vitro.

**Materials and methods:** Isolated rat islets were incubated over periods of 48 hours with increasing concentrations of individual amino acids (0.1 μM–10 mM). The amino acids selected for investigation were those with known insulin stimulatory effects (L-arginine, L-alanine, L-glutamine), and the branched chain amino acids (L-valine, L-leucine, L-isoleucine). After the chronic incubation period islets were assessed for beta cell proliferation and insulin secretion capacity.

**Results:** The results showed that while no amino acid tested had beta cell protective effects, some showed beta cell toxicity including insulinotropic amino acids L-alanine and L-arginine and branched chain amino acid L-valine. L-alanine and L-valine significantly decreased glucose stimulated insulin secretion ( $-22\% \pm 5.3\%$ ,  $p < 0.05$ ) at elevated 10 mM levels. The amino acid L-arginine was particularly beta cell toxic causing a dose dependant decrease in insulin secretion becoming significant at 1 mM with a 75% reduction at 10 mM ( $p < 0.01$ ). This was accompanied by a significant decrease in beta cell proliferation as measured by EdU incorporation ( $-30\%$  at 1 mM,  $p < 0.05$ ) and increased beta cell apoptosis as measured by annexin V. We hypothesised that arginine induced production of nitric oxide was the mechanism underlying beta-cell dysfunction however its effects were not blocked by NOS inhibitors L-NAME and Aminoguanidine, and beta cell nitrite levels and iNOS expression remained unchanged. Dysregulated cytosolic calcium concentration is a potent signal for apoptosis and basal calcium concentrations were found to be significantly elevated in L-arginine treated beta cells ( $p < 0.05$ ), indicating that sustained calcium influx may be the mechanism responsible its toxic effect.

**Conclusion:** In conclusion these results show for the first time that altered amino acid levels in obesity may impact on beta cell function providing a further link between obesity and type 2 diabetes.

Supported by: IRCSET

522

### MST1 mediates beta cell failure by targeting BIM and destabilising PDX1

A. Ardestani<sup>1</sup>, F. Paroni<sup>1</sup>, J. Kerr-Conte<sup>2</sup>, K. Maedler<sup>1</sup>;<sup>1</sup>Centre for Biomolecular Interactions, University of Bremen, Germany,<sup>2</sup>University of Lille, Lille, France.

**Background and aims:** Pancreatic β-cell death is the fundamental cause of type 1 and type 2 diabetes. Mammalian sterile 20-like kinase 1 (MST1) is a serine threonine kinase, which mediates apoptosis in response to cytotoxic stress. MST1 is both, cleaved and activated by caspases, and in turn activates caspases to amplify the apoptotic signaling pathways. MST1 is induced in β-cells under diabetic conditions, which may be a major common pathway of β-cell death in diabetes. Here we examined the mechanism by which MST1 mediates β-cell failure.

**Materials and methods:** Isolated human islets and the rat β-cell line INS1 were exposed to a diabetic milieu (IL-1β/IFNγ, H<sub>2</sub>O<sub>2</sub> or increasing glucose concentrations). Phospho-MST1 and MST1 cleavage, Pancreatic Duodenal Homeobox-1 (PDX1), the apoptotic mitochondrial pathway and β-cell apoptosis (caspase-3, -9 & PARP) were analyzed by western blotting. MST1 was overexpressed by adenoviral-delivery or plasmid transfection, MST1 inactivation was performed by overexpressing dominant-negative MST1 (dn-MST1) or a stable MST1-deficient INS1 cell clone was produced by lentiviral

MST1 small hairpin RNA (shRNA) transfection. Lentiviral MST1 shRNA and pools of specific siRNA was also used to knock down MST1 in human islets. PDX1 turnover & ubiquitination was performed in HEK 293 cells over-expressing PDX1 and MST1.

**Results:** MST1 cleavage & phosphorylation was increased in human islets and INS1 cells exposed to IL-1β/IFNγ, H<sub>2</sub>O<sub>2</sub> or increasing glucose concentrations (22.2 and 33.3 mM). This correlated with increased β-cell apoptosis, impaired function, decreased PDX1, BCL2 and increased BAX, BIM, cytochrome c release and activation of caspase-9 and -3, indicating activation of the mitochondrial pathway. Overexpression of MST1 increased β-cell apoptosis and reduced GSIS, indicating that MST1 alone is sufficient to promote β-cell failure. The intrinsic cell death pathway as a mediator of MST1-induced β-cell apoptosis was confirmed by co-culture with BAX inhibitory peptide, which diminished caspase cleavage (9 and 3) and apoptosis. MST1 overexpression in islets, INS-1 cells as well as in PDX1 overexpressing HEK293 cells strongly decreased PDX1 without changes in PDX1 mRNA levels; this demonstrated that the decrease in PDX1 expression was regulated at the post-transcriptional level and related to its reduced stability. MST1 overexpression caused PDX1 shuttling from the nucleus to the cytosol, its ubiquitination and subsequent degradation, which occurred by direct MST1-induced phosphorylation of PDX1 through physical interaction of with MST1. MST1-induced PDX1 degradation was inhibited by the proteasome inhibitor MG132, indicating ubiquitin-proteasome mediated PDX1 proteolysis. MST1-knock down in human islets and INS-1 cells protected β-cells from gluco- and cytokine-toxicity; it improved survival as well as β-cell function through downregulation of BIM and restoration of PDX1, indicating a major role of BIM and PDX1 in the mechanism of stress-induced beta cell apoptosis.

**Conclusion:** Our results show that MST1 plays an important role in triggering β-cell death via targeting proapoptotic BIM and destabilizing prosurvival signal PDX1. Its inhibition provides a new strategy to restore β-cell survival and function.

Supported by: JDRF

523

### Identification of new mitochondrial molecular targets in INS-1E beta cells exposed to metabolic stresses associated with diabetes

T. Brun<sup>1</sup>, D. Duhamel<sup>1</sup>, P. Scarica<sup>2</sup>, P. Gaudet<sup>3</sup>, A. Bairochi<sup>3</sup>, F. Palmieri<sup>2</sup>, P. Maechler<sup>1</sup>;<sup>1</sup>Department of Cell Physiology and Metabolism, University of Geneva,Switzerland, <sup>2</sup>Department of Pharmacology-Biology, University of Bari, Italy,<sup>3</sup>Swiss Institute of Bioinformatics, University of Geneva, Switzerland.

**Background and aims:** Exposure of beta-cells to stress conditions, such as high glucose, fatty acids and oxidative stress, interrupts the transduction of signals normally coupling glucose metabolism to insulin secretion, mitochondria playing a central role in this process. Several questions remain open on the respective contributions of the different diabetes-associated stresses and accompanying beta-cell dysfunction. Here, we aimed at identifying putative molecular targets shared by the different metabolic stresses investigated in the same study using new powerful expression profiling approach.

**Materials and methods:** We designed a molecular screening array (Mito-array) based on pre-loaded micro fluidic card Real Time PCR system. INS-1E cells were cultured for 3 days in different conditions: 1) basal 5.6 mM glucose 2) intermediate 11.6 mM glucose 3) high 25 mM glucose 4) 0.4 mM palmitate 5) 0.4 mM oleate 6) after transient oxidative stress (200 μM H<sub>2</sub>O<sub>2</sub> for 10 min). The Mito-array allowed analysis of 60 selected genes with a particular interest for mitochondrial carriers of the Slc25a family. Molecular targets of interest have been further evaluated at the protein level by immunoblotting. The expression profile was then coupled with bio-informatics tools to delineate comprehensive networks.

**Results:** We first validated our gene expression data with selected markers previously identified in each specific condition. The expression of several energy sensors, key transcription factors and metabolic related enzymes were markedly diminished by high glucose (*Ampk*, *Pdx-1*, *Gk*), oxidative stress (*Pgc1a*, *Tfam*, *Cs*, *Mdh2*) or both (*Sirt1*, *Sirt4*, *Mafa*, *Ppara*, *PC*); whereas exposure to fatty acids did not alter their transcript and protein levels. High glucose and oxidative stress promoted cell death through caspase 3 cleavage activation, while palmitate induced ER stress without causing apoptosis. Among the 60 genes of interest investigated by Mito-array, we identified 22 mitochondrial carriers of the Slc25a family. High glucose selectively up-regulated protein levels of the citrate/isocitrate carrier (CIC), the dicarboxylate transporter (DIC) and the glutamate transporter (GC1). This is compatible with high activities of mitochondrial anaplerotic/cataplerotic pathways and

NADPH-generating shuttles. Both fatty acids increased expression of the carnitine/acylcarnitine carrier (CAC).

**Conclusion:** Expression of mitochondrial solute carriers was altered selectively by the different metabolic stresses, exhibiting stress-specific signatures. Due to the complexity of the alterations induced by the different stresses and the number of genes investigated, an innovative approach using bioinformatics tools is proposed, in order to obtain a comprehensive view of these results.

*Supported by: SNF, De-N Yde Foundation*

## 524

### Quantification of hydrogen peroxide generation within the endoplasmic reticulum by using the $H_2O_2$ -sensitive biosensor HyPer in insulin-producing cells

I. Mehmeti, S. Lortz, S. Lenzen;

Institute of Clinical Biochemistry, Hannover Medical School, Germany.

**Background and aims:** Oxidative protein folding in the endoplasmic reticulum (ER) is associated with the formation of native disulfide bonds. This process is catalysed by thiol-disulfide oxidoreductases PDI (protein disulfide isomerase) and ERO-1 (ER oxidoreductin 1) inevitably leading to hydrogen peroxide ( $H_2O_2$ ) generation. Especially in cells with very high secretory activity, such as pancreatic  $\beta$ -cells, the  $H_2O_2$  molecules generated during the oxidative protein folding could represent a significant oxidative burden. Therefore the aim of this study was to quantify the  $H_2O_2$  generation during disulfide bond formation in living insulin-producing cells by targeting and specifically expressing the  $H_2O_2$ -sensitive biosensor HyPer into the ER.

**Materials and methods:** For quantification of compartment-specific  $H_2O_2$  concentrations in living insulin-producing cells, the  $H_2O_2$ -sensitive biosensor HyPer was specifically expressed in cytosol (Cyto-HyPer), peroxisomes (Peroxi-HyPer), mitochondria (Mito-HyPer) or in the ER (ER-HyPer). The changes of the HyPer fluorescence ratio, which represents the  $H_2O_2$  concentration, were assessed fluorometrically and microscopically. The redox state of HyPer in various cell organelles was analysed by treatment of the HyPer expressing cells with 0.5 mM dithiothreitol (DTT). Additionally, the ER-resident  $H_2O_2$ -metabolizing peroxiredoxin 4 (PRDX4) was stably overexpressed to determine the specificity of the HyPer protein for the quantification of  $H_2O_2$  within the ER.

**Results:** Stable expression of the  $H_2O_2$ -sensitive HyPer protein targeted to various cell organelles revealed that the fluorescence ratio of HyPer in the ER was significantly higher than in all other investigated cell organelles, indicating high basal  $H_2O_2$  concentrations in this organelle. Treatment of the Cyto-, Peroxi-, Mito- and ER-HyPer expressing cells with the disulfide-bond-reducing agent DTT resulted in an extreme decrease of the HyPer fluorescence ratio in the ER, suggesting that ER-HyPer is completely oxidized. However, the overexpression of the ER-resident  $H_2O_2$ -metabolizing peroxiredoxin 4 had no impact on the observed ER-HyPer fluorescence ratio, although the overexpression of PRDX4 successfully prevented  $H_2O_2$ -mediated toxicity.

**Conclusion:** These data provide evidence that the oxidation of the  $H_2O_2$ -sensitive HyPer protein in the ER solely reflects the ER luminal oxidizing thiol-disulfide milieu and not the  $H_2O_2$  generation. Thus, the HyPer protein is an unsuitable tool for  $H_2O_2$  monitoring during disulfide bond formation in the ER. Furthermore the results clearly show on the other side that overexpression of ER-resident peroxiredoxin 4 is able to protect insulin-producing cells against oxidative injury when exposed to external  $H_2O_2$ , indicating that PRDX4 is a potential  $H_2O_2$  scavenger within the ER.

## 525

### Pancreatic beta cell proliferation by intermittent hypoxia via up-regulation of Reg family genes

S. Takasawa<sup>1</sup>, H. Ota<sup>2</sup>, A. Itaya-Hironaka<sup>1</sup>, A. Yamauchi<sup>1</sup>, S. Sakuramoto-Tsuchida<sup>1</sup>, T. Miyaoka<sup>1</sup>, T. Fujimura<sup>1</sup>, H. Tsujinaka<sup>1</sup>, K. Yoshimoto<sup>1</sup>, S. Tamaki<sup>1</sup>, H. Kimura<sup>2</sup>;

<sup>1</sup>Biochemistry, <sup>2</sup>Internal Medicine, Nara Medical University, Kashihara, Japan.

**Background and aims:** Sleep apnea syndrome (SAS) is characterized by recurrent episodes of oxygen desaturation during sleep, the development of daytime sleepiness, and deterioration in the quality of life. Up to 30% of the adult populations in Western countries are thought to be affected by asymptomatic SAS and approximately 2-4% by symptomatic SAS. Accumulating evidence suggests the association of intermittent hypoxia (IH), a hallmark

of SAS, and type2 diabetes. The progression to type 2 diabetes is dependent on the compensatory responses of the pancreatic  $\beta$  cells that produce insulin. Hyperglycemia is known to increase the  $\beta$  cell replication, and therefore IH itself could also modify the ability of the pancreatic  $\beta$  cells to replicate. However, we have currently no knowledge of the direct impact of IH on pancreatic  $\beta$  cell proliferation. In the present study, using rat, hamster, and human pancreatic  $\beta$  cells, we investigated the direct effect of IH on  $\beta$  cell proliferation.

**Materials and methods:** Pancreatic  $\beta$  cell-derived cell lines (rat RINm5F cells, hamster HIT-T15 cells and human 1.1B4 cells) were exposed either to 64 cycles/24 h of IH (5 min hypoxia (1%  $O_2$ )/10 min normoxia (21%  $O_2$ )), sustained hypoxia (SH: 1%  $O_2$ ) or normoxia for 24 h. After the treatment, viable cell numbers and apoptosis were measured by WST-8 cleavage and TUNEL method, respectively. Real-time quantitative RT-PCR of regenerating gene (Reg) family genes (Reg I, PAP I/Reg2, PAP II/Reg III, PAP III, and Reg IV for rat, and REG Ia, REG Ib, REG III, HIP/PAP, and REG IV for human), and interleukin-6 (IL-6) was performed using normoxia-, IH-, or SH-treated RINm5F and 1.1B4 cell RNAs as template. Small interfering RNA (siRNA) for rat Reg family were introduced into RINm5F cells and the IH-induced RINm5F cell proliferation was measured by WST-8 cleavage assay.

**Results:** The cell numbers of HIT-T15, RINm5F and 1.1B4 cells were significantly increased by IH ( $P=0.0064$ ,  $0.0013$ , and  $0.013$ , respectively), whereas SH did not increase the numbers of these cells. IH did not increase the apoptosis of the  $\beta$  cells, suggesting that IH stimulates  $\beta$  cell proliferation. We next measured the mRNA levels of Reg family genes, which encode autocrine  $\beta$  cell growth factors, and found that Reg I, PAP II/Reg III, PAP III, and Reg IV mRNAs were significantly increased in rat RINm5F cells by IH and that REG Ia mRNA was specifically increased in human 1.1B4  $\beta$  cells by IH but not by SH. The knockdown experiments using rat Reg family gene siRNAs for Reg I, PAP II/Reg III, PAP III and Reg IV resulted in reduced IH-induced cell proliferation of RINm5F cells but the introduction of siRNA for PAP I/Reg2 did not change the IH-induced RINm5F cell proliferation. In addition, IH significantly increased the mRNA level of IL-6, which was reported to increase Reg family gene expression, in RINm5F and 1.1B4 cells.

**Conclusion:** The cyclic change of hypoxia-reoxygenation, which occurs in SAS patients, stimulates pancreatic  $\beta$  cell proliferation. In this process, up-regulation of Reg family genes could be involved. The IH-induced expression of IL-6 may be a trigger for the up-regulation of Reg family genes.

*Supported by: JSPS KAKENHI*

## 526

### Hypoxia modifies intracellular free $Zn^{2+}$ concentration in pancreatic beta cells by altering the expression of the metallothionein and zinc transporter genes Slc30a8 / Slc39a6

P.A. Gerber, E.A. Bellomo, G.A. Rutter;

Section of Cell Biology, Department of Medicine, Imperial College, London, UK.

**Background and aims:** The endocrine pancreas is one of the best vascularised tissues, and insufficient supply with oxygen impairs the function of pancreatic islets. This is of particular importance in the setting of islet isolation and transplantation, but there is also evidence that hypoxia regulates islet function in type 2 diabetes (T2D) as well as in a low-oxygenated subpopulation of islets that form a functional reserve of endocrine cells.  $Zn^{2+}$  is an important cofactor for insulin biosynthesis and storage in pancreatic  $\beta$ -cells and is tightly controlled by transporters and binding proteins including the T2D-associated gene Slc30a8 (ZnT8). Here, we explore the effects of hypoxia on the regulators of  $Zn^{2+}$  homeostasis.

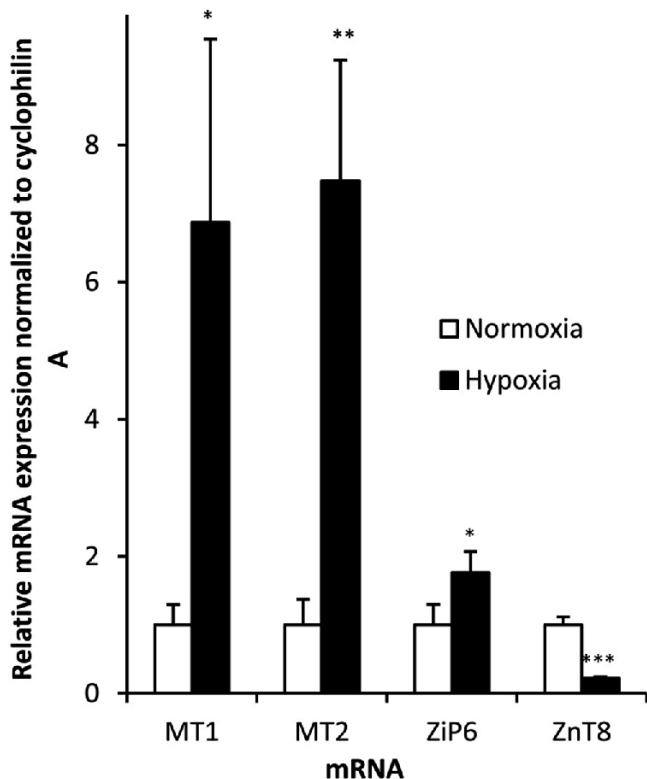
**Materials and methods:** Isolated murine pancreatic islets as well as dispersed islet cells were exposed to hypoxia (1% oxygen) or normoxia (21% oxygen) for 24 hours. After exposure, mRNA was isolated and quantified relatively to the expression of cyclophilin by qRT-PCR. The cytosolic concentration of free zinc ( $[Zn^{2+}]_{cy}$ ) in dispersed islet cells was measured using our newly developed FRET-based  $Zn^{2+}$  probe eCALWY-4, which was delivered to cells by adenoviral transfection.

**Results:** After 24 hours of hypoxia exposure of intact isolated islets, the gene expression of the metallothionein 1 and 2 genes (MT1, MT2) was up-regulated by a factor of  $6.9 \pm 2.7$  ( $p < 0.05$ ) and  $7.5 \pm 1.8$  ( $p < 0.01$ ), respectively. The expression of the zinc transporter ZiP6, encoded by Slc39a6, was increased by  $1.8 \pm 0.3$  ( $p < 0.05$ ), whereas the expression of ZnT8, encoded by Slc30a8, was decreased to  $0.2 \pm 0.0$  ( $p < 0.001$ ) compared to islets that were cultured under normoxic conditions (figure). To a lesser extent, similar changes were observed in dispersed islet cells after exposure to hypoxia (fold induction compared to cells under normoxic conditions: MT1  $2.5 \pm 0.1$ , MT2  $3.0 \pm 0.2$ ,



ZiP6  $1.6 \pm 0.2$ , ZnT8  $0.5 \pm 0.1$ ;  $p < 0.05$  for all measurements).  $[Zn^{2+}]_{\text{cyt}}$  tended to be decreased after exposure to hypoxia for 24 hours when compared to normoxic islets ( $309 \pm 54 \text{ pmol/l}$  vs.  $425 \pm 284 \text{ pmol/l}$ ,  $n=3$ ).

**Conclusion:** We demonstrate that exposure to hypoxia up-regulates metallothionein gene expression and alters the expression of 2 zinc transporters with very high abundance in the pancreatic  $\beta$ -cell, ZiP6 and ZnT8. Whereas an increase in metallothionein expression may decrease  $[Zn^{2+}]_{\text{cyt}}$ , and increased ZiP6 and decreased ZnT8 activity would promote an increase in  $[Zn^{2+}]_{\text{cyt}}$ . The concentration of  $[Zn^{2+}]_{\text{cyt}}$  in dispersed islet cells tended to decrease after 24 hours of hypoxia exposure, but further investigation is necessary to dissect the impact of the altered metallothionein and zinc transporter gene expression levels on cytosolic zinc concentrations under hypoxia. Changes in intracellular zinc homeostasis are thus involved in the response of  $\beta$  cells to hypoxia and might be a target for strategies aimed at improving cell survival *in vitro* prior to transplantation.



Supported by: Swiss Life Foundation

## 527

**Proinsulin homeostasis in beta cells is sensitive to oxidative stress and its disorder and toxic consequences can be alleviated by administration with antioxidants**

Q. Yuan<sup>1,2</sup>, K. Osei<sup>1</sup>, J. Wang<sup>1</sup>;

<sup>1</sup>Division of Endocrinology, Diabetes, and Metabolism, Department of Internal Medicine, The Ohio State University, Columbus, USA,

<sup>2</sup>Endocrinology and Metabolism, The First Affiliated Hospital of Nanjing Medical University, China.

**Background and aims:** Proinsulin with a low relative folding rate maintains a homeostatic balance of natively and plentiful non-natively folded states (i.e., proinsulin homeostasis, PIHO) in  $\beta$ -cells. Also, findings in our recent study have suggested that the contrast of low relative folding rate with plentiful amounts of insulin precursor manufactured in  $\beta$ -cells renders PIHO susceptible to genetic and environmental influences, and a disturbed PIHO would result in various toxic consequences that critically link to  $\beta$ -cell failure and diabetes. To explore this hypothesis, we further characterized the susceptibility of PIHO to oxidative stress and the effect of antioxidants on PIHO disorder and toxic consequences in  $\beta$ -cells.

**Materials and methods:** Oxidative stress inducer hydrogen peroxide ( $H_2O_2$ ) and/or antioxidants including dithiothreitol, glutathione, N-acetylcysteine, and vitamin C were applied in this study in the *Ins2<sup>+/Akita</sup>* (C96Y)  $\beta$ -cells that

preserve an oxidative stress consequence and wild-type control *Ins2<sup>+/+</sup>*  $\beta$ -cells. We applied immunoblotting, immunoprecipitation, and morphological studies and examined changes in the cellular proinsulin states, secreted insulin, and death rates of these two line  $\beta$ -cells under oxidative stress and with/without antioxidant conditions. Statistical analyses are performed using Student's t-test (2-tailed) or analysis of variance if appropriate, with  $P < 0.05$  considered statistically significant.

**Results:** An increase in the proportion of non-natively folded proinsulin aggregation in *Ins2<sup>+/+</sup>*  $\beta$ -cells was induced by addition of  $H_2O_2$  at low nanomolar concentrations during a 60-minute post-translational process compared to the control with no addition of  $H_2O_2$  ( $P < 0.01$ ). Correspondingly, a decrease was evident in the proportion of fully folded proinsulin monomers and in the consequently secreted insulin. These changes were induced by  $H_2O_2$  in a dose-dependent manner. Moreover, addition of 10  $\mu M$  but not 1  $\mu M$   $H_2O_2$  for 24 or 48 hours induced an increased cell death compared to the control with no addition of  $H_2O_2$  ( $P < 0.05$ ) despite addition of 10  $\mu M$  or 1  $\mu M$   $H_2O_2$  all induced a disorder in the PIHO of *Ins2<sup>+/+</sup>*  $\beta$ -cells. Intervention with the above mentioned antioxidants partially eliminated the PIHO disorder and toxic consequences in insulin secretion and cell survival, which were primarily induced by  $H_2O_2$  in *Ins2<sup>+/+</sup>*  $\beta$ -cells and by the C96Y mutation in the *Ins2* gene in *Ins2<sup>+/Akita</sup>*  $\beta$ -cells.

**Conclusion:** The findings in this study clearly demonstrated that proinsulin homeostasis in normal  $\beta$ -cells is sensitive to oxidative stress because perturbation of PIHO can be induced by  $H_2O_2$  at nanomolar concentrations but this level of  $H_2O_2$  is unable to induce  $\beta$ -cell death. The data also suggest that administration with antioxidants can alleviate PIHO disorders and toxic consequences that associate to oxidative stress in  $\beta$ -cells.

## 528

**Glucagon-like peptide-1 induces antioxidant enzymes via extracellular regulated kinase pathway in beta cells**

E. Fernández-Millán<sup>1,2</sup>, J. De Toro-Martín<sup>1</sup>, E. Lizárraga-Mollinedo<sup>1,2</sup>, S. Ramos<sup>3</sup>, L. Goya<sup>3</sup>, F. Escrivá<sup>1,2</sup>, M.A. Martín<sup>3</sup>, C. Álvarez<sup>1,2</sup>;

<sup>1</sup>Bioq. y Biol. Mol. II, Facultad de Farmacia, UCM, <sup>2</sup>Ciberdem, ISCIII,

<sup>3</sup>Instituto de Ciencia y Tecnología de Alimentos y Nutrición, CSIC, Madrid, Spain.

**Background and aims:** One of the major factors for progressive loss of  $\beta$ -cell function and mass in type 2 diabetes is glucotoxicity. Long-term hyperglycemia induces massive generation of reactive oxygen species (ROS) leading to chronic oxidative stress.  $\beta$ -cells are extremely sensitive to oxidative stress because of their low levels of antioxidant enzymes. Redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a pivotal role in the cellular defence against oxidative stress via transcriptional upregulation of phase II defence enzymes and antioxidant stress proteins. The incretin hormone glucagon-like peptide-1 (GLP-1) protects  $\beta$ -cells from oxidative stress-induced apoptosis both *in vivo* and *in vitro*. However the signalling pathways involved are not fully defined. To gain insight into the mechanisms by which GLP-1 protects  $\beta$ -cells from oxidative stress-derived effects we have used an *in vitro* model of oxidative stress induced by tert-butyl hydroperoxide (*t*-BOOH).

**Materials and methods:** INS-1E cells treated for 20h with GLP-1 were further exposed to *t*-BOOH for 2h and ROS generation at different times (5–120 min) and cell death at 2h were measured by 2',7'-dichlorofluorescein (DCFH) and crystal violet assays, respectively. The content of reduced glutathione (GSH) was quantified by a fluorometric assay and glutathione peroxidase (GPx) and glutathione reductase (GR) activities were analyzed indirectly. To elucidate the upstream signalling pathways involved in the activation of GR and GPx, specific inhibitors were used. Finally, to study the effect of GLP-1 on Nrf2 translocation, nuclear and cytosolic extracts were obtained and WB against Nrf2 was performed.

**Results:** Pre-treatment of cells with GLP-1 significantly reduced the generation of ROS caused by *t*-BOOH and suppressed the deleterious effect induced by the pro-oxidant. Since induction of antioxidant defences is considered crucial to protect cells against oxidative injuries we evaluated the effect of GLP-1 treatment on the activity of GPx and GR enzymes. Long-term treatment of GLP-1 enhanced GPx and GR activities and GSH content in a dose-dependent manner. The inhibition of PKA and Erks completely blocked the cytoprotective effect of GLP-1 whereas no effect was observed when Akt was inhibited. GLP-1 also induced Nrf2 transcription factor activation. Pre-treatment of cells with GLP-1 for 3, 6 and 20h, increased the protein levels of Nrf2 in the nucleus at 3 h, peaked at 6 h and then declined at 20 h of treatment.

**Conclusion:** Herein we show that GLP-1 is able to enhance the cellular antioxidant defence capacity of  $\beta$ -cells, by increasing the activity of GPx and GR enzymes via a mechanism that involves activation of Erks in a PKA-dependent manner and translocation of Nrf2. In conclusion, our results provide an additional mechanism of action of GLP-1 to prevent oxidative damage in  $\beta$ -cells through the modulation of signalling pathways involved in antioxidant enzyme regulation.

*Supported by: MINECO (BFU 2011-25420), CIBERDEM (ISCIII), Spain*

## 529

### Increasing expression of UCP2 in mouse beta cells partially prevents secretory dysfunction induced by glucotoxic conditions and oxidative stress

N. Li, F. Assimacopoulos-Jeannet, P. Maechler;

Department of Cell Physiology and Metabolism, University of Geneva, Switzerland.

**Background and aims:** The function of uncoupling protein 2 (UCP2) in pancreatic beta-cells is debated, both in terms of putative mitochondrial uncoupling properties and effects on glucose-stimulated insulin secretion. In this context, recent studies suggest a protective role for UCP2 in stress conditions. Here, we questioned protective capacity of UCP2 under oxidative stress and glucotoxic culture conditions using transgenic mice with beta-cell-specific overexpression of human UCP2 (RIP-UCP2).

**Materials and methods:** Islets isolated from littermate wild type (WT) control and RIP-UCP2 mice were challenged with single transient oxidative stress (200  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 10 min) and analysed either right after stress (acute stress) or after a recovery culture period (3 days post-stress). Alternatively, glucotoxicity was induced by culturing islets with 30mM glucose (versus 11mM for standard culture) for 7 days. We measured glucose-induced insulin secretion (RIA), UCP2 protein expression (immunoblotting), mitochondrial membrane potential (rhodamine-123), intracellular superoxide generation (DHE), and apoptosis (ELISA).

**Results:** In control no-stress conditions, RIP-UCP2 islets exhibited similar secretory responses to 22.8mM glucose compared to WT islets. In acute oxidative stress conditions, i.e. immediately after 10min exposure to H<sub>2</sub>O<sub>2</sub>, both WT and RIP-UCP2 islets totally lost the glucose response. Three days after transient oxidative stress, the rate of insulin secretion at stimulatory 22.8mM glucose in WT and RIP-UCP2 islets was back to normal. However, basal release at 2.8mM glucose was 1.7-fold higher in WT islets compared to non stressed islets, while RIP-UCP2 islets exhibited normal low basal secretion (-51%,  $p < 0.05$  versus WT). In glucotoxic conditions, i.e. after 7 days culture at 30mM glucose, basal insulin release and glucose-stimulated secretion were increased in WT islets compared to 11mM glucose conditions (12- and 4.2-fold respectively, both  $p < 0.01$ ), thereby reducing the glucose response. RIP-UCP2 islets maintained in glucotoxic conditions exhibited lower basal and stimulated insulin secretion rates (-56% and -40% respectively versus WT islets,  $p < 0.001$ ), partially preserving the glucose response. No mitochondrial uncoupling was observed in RIP-UCP2 islets when cultured at 11mM glucose. In glucotoxic conditions, RIP-UCP2 islets showed partially preserved glucose-induced mitochondrial hyperpolarisation, which was markedly impaired in WT islets (-42% in WT and -15% in RIP-UCP2 islets,  $p < 0.05$ ). Preliminary data indicated similar superoxide production and cell death in WT and RIP-UCP2 islets.

**Conclusion:** UCP2 did not exhibit uncoupling properties. Increasing beta-cell UCP2 partially protected against glucotoxic conditions and oxidative stress, mainly by preserving low basal insulin release and mitochondrial glucose response.

*Supported by: SNF*

## 530

### Expression of the TXNIP gene is influenced by histone modifications in INS1 832/13 cells

M. Cai<sup>1</sup>, O. Kotova<sup>2</sup>, Y. de Marinis<sup>1</sup>, L. Groop<sup>1,3</sup>;

<sup>1</sup>Department of Clinical Science, Diabetes and Endocrinology, Lund University, Skåne University Hospital, Malmö, Sweden, <sup>2</sup>Department of Clinical Science, Lund University, Skåne University Hospital, Malmö, Sweden, <sup>3</sup>Institute for Molecular Medicine Finland FIMM, University of Helsinki, Finland.

**Background and aims:** Thioredoxin-interacting protein (TXNIP) has been identified as a mediator of glucotoxicity in pancreatic B cells, but the mechanisms are not fully understood. High glucose has been suggested to increase binding of a carbohydrate-response element-binding protein (ChREBP) to TXNIP promoter, but it could also influence the chromatin. This study was designed to test whether glucose can influence TXNIP expression by causing histone modifications.

**Materials and methods:** We treated INS1 832/13 cells with 5 mM, 16.7 mM and 25 mM glucose for 4 h and measured TXNIP mRNA expression by quantitative RT-PCR. Histone modifications were analyzed by Chromatin immunoprecipitation (ChIP) using antibodies against H3K9 acetylation, H3K4me3 and H3K27me3.

**Results:** Incubating INS1832/13 cells with 16.7 mM and 25 mM glucose increased TXNIP mRNA 4.6-fold ( $p = 0.0019$ ) and 5.8-fold ( $p = 0.0000$ ), respectively, as compared to expression at 5 mM glucose. The same glucose concentrations also induced a 2.6-fold and 3.6-fold increase in H3K9 acetylation (both  $p = 0.04$ ). High glucose (25 mM) increased a 1.76-fold trimethylation at H3K4 ( $p = 0.04$ ) and decreased trimethylation (-35.35%) at H3K27 ( $p = 0.04$ ). There was a strong positive correlation between H3K9 acetylation at 16.7 mM glucose and increase in TXNIP expression ( $r = 0.831$ ;  $p = 0.04$ ).

**Conclusion:** Glucose induced increase in H3K9 acetylation may contribute to increased TXNIP expression in clonal beta-cells and thereby contribute to glucotoxicity.

## PS 031 Beta cell apoptosis and survival

531

### Glucose-induced ER stress in beta cells induces CHOP-mediated activation of the intrinsic apoptosis pathway

J.A. Wali<sup>1,2</sup>, T.W. Kay<sup>1,2</sup>, H.E. Thomas<sup>1,2</sup><sup>1</sup>St Vincent's Institute, <sup>2</sup>University of Melbourne, Melbourne, Australia.

**Background and aims:** Type-2 diabetes is caused by declining function and increased apoptosis of beta-cells in insulin-resistant subjects. Chronic hyperglycemia is considered a potential cause of this beta-cell death. High glucose concentrations induce apoptosis of islet cells *in vitro* through the intrinsic pathway. Specifically, the pro-apoptotic BH3-only proteins Bim and Puma are required. We studied intrinsic pathway activation in response to glucose and ribose-induced ER stress.

**Materials and methods:** Islets isolated from wild-type (B6), Bim<sup>-/-</sup>, Puma<sup>-/-</sup> or CHOP<sup>-/-</sup> mice were cultured for 4–6 days in 33.3 mM glucose or 50 mM ribose (a reducing sugar similar to glucose), together with the ER stress inhibitors tauroursodeoxycholic acid (TUDCA 0.5 mM) or phenylbutyric acid (PBA 2.5 mM). Islets were incubated with Thapsigargin (5  $\mu$ M) for 1 or 5 days. Apoptosis was measured by flow cytometric analysis and ER stress factors were studied in ribose treated (1–3 days) islets by qPCR and western blotting. **Results:** Bim<sup>-/-</sup> and Puma<sup>-/-</sup> mice had normal glucose tolerance, and glucose stimulated insulin secretion (GIS) of isolated islets was similar to wild-type mice. This suggested that Bim and Puma deficient islets are functionally normal. Ribose treatment of wild-type, Bim or Puma deficient islets produced a similar decrease in GIS. Loss of Bim or Puma partially protected islets from Thapsigargin toxicity indicating that the intrinsic apoptosis pathway is involved in ER stress induced cell death. Inhibition of ER stress with TUDCA or PBA partially protected wild-type islets, and completely protected Bim<sup>-/-</sup> or Puma<sup>-/-</sup> islets from ribose and glucose toxicity. This showed that Bim and Puma play overlapping and additive roles in mediating this apoptosis. Ribose treatment for 3 days increased JNK phosphorylation and XBP1 splicing (1.73 $\pm$ 0.10 fold) but did not increase mRNA of ER chaperones (BiP, Pdia4, P58), or BiP protein expression in wild-type islets. Gene and protein expression of the ER stress transcription factor CHOP was significantly increased (1.6 $\pm$ 0.08 fold) in ribose-treated wild-type islets compared with untreated islets, and islets deficient in CHOP were partially protected from ribose and glucose toxicity. These results suggest there is activation of the IRE1 $\alpha$  (p-JNK, XBP1s) and PERK (CHOP) arms, and inactivation of the ATF6 arm (chaperones) of the ER stress pathway in ribose treated islets. CHOP and BiP mRNA expression was significantly lower in islets of Bim<sup>-/-</sup> mice (CHOP: 0.54 $\pm$ 0.12 fold), and to a lesser extent in islets of Puma<sup>-/-</sup> mice. Incubation of wild-type islets with ribose+TUDCA reduced gene expression of CHOP (0.8 $\pm$ 0.10 fold) and upstream factor ATF4 (0.56 $\pm$ 0.18 fold). Gene expression of Bim and Puma also decreased after TUDCA exposure (Ribose vs Ribose+TUDCA). These results suggested that down-regulation of CHOP protects islets from cell death mediated by the intrinsic apoptosis pathway.

**Conclusion:** Our results suggest that ER stress plays an important role in glucose and ribose-induced killing of islet-cells *in vitro*. Glucose toxicity activates the pro-apoptotic factors in the ER stress pathway including CHOP, JNK and XBP1, and this activates the intrinsic apoptosis factors Bim and Puma. Up-regulation of the upstream ER stress transcription factor CHOP is important in mediating intrinsic pathway activation in islets in response to ribose and glucose toxicity.

Supported by: NHMRC Australia

532

### Losartan protects human pancreatic islets from glucotoxicity by interfering with the activation of the phospholipase C (PLC)/Calcium signalling pathway

R. Cassel<sup>1</sup>, S. Ducreux<sup>2</sup>, S. Dubois<sup>1</sup>, G. Vial<sup>1</sup>, M.-A. Chauvin<sup>1</sup>, J. Riusset<sup>1</sup>, F. Van Coppenolle<sup>2</sup>, C. Thivolet<sup>1</sup>, A.-M. Madec<sup>1</sup><sup>1</sup>Faculté de Médecine Lyon Sud, CarMeN Inserm U1060, Oullins <sup>2</sup>Université Claude Bernard Lyon1, UMR CNRS 5534, Lyon, France.

**Background and aims:** Chronic high glucose (HG) concentrations induce beta cell injury and contribute to the progressive incapacity of glucose control in patients with type 2 diabetes. As potential mechanisms, HG levels increase

reactive oxygen species (ROS) production and induce endoplasmic reticulum (ER) stress leading to beta cell death by apoptosis and necrosis. We have previously shown that Losartan, an angiotensin II Type-1 receptor (AT1R) blocker, reduces the deleterious effects of HG on human islets by reducing oxidative and ER stress. The aim of this study was to investigate the mechanisms of the protective effects of Losartan on mitochondrial function and calcium homeostasis in human islets and murine beta cell line exposed to HG conditions.

**Materials and methods:** Human islets from 11 distinct donors were incubated for 96h in 5.5 or 16.7 mM glucose with or without Losartan for the last 48h, and/or with acute or chronic U-73122, a PLC inhibitor. MIN6B1 beta cells were cultured 48h in 5.5 mM or 16.7 mM with or without Losartan and/or PLC inhibitor the last 24h. ATP5B (ATP synthase mitochondrial) and UCP2 mRNA were evaluated by qRT-PCR. OxPhos, SERCA2b (sarco/endoplasmic reticulum ATPase) and IP3R2 (ER-resident inositol triphosphate receptors) proteins were measured by western-blot. Cytosolic calcium [Ca<sup>2+</sup>]<sub>i</sub> was measured in Ca<sup>2+</sup> free KRB on human islets loaded with Fura-2 AM. Thapsigargin, a SERCA pump blocker, was used to release reticular calcium [Ca<sup>2+</sup>]<sub>ER</sub>.

**Results:** In human islets, UCP2 mRNA level increased in HG conditions (x2.5;p<0.05), while Losartan prevented this effect (p<0.05). HG induced a significant reduction in mitochondrial respiratory chain complex I protein levels (p<0.01), complex III (p<0.05) and Complex V (p<0.05) as compared to control islets. Addition of Losartan up-regulated complex I (p<0.01) and complex V (p<0.05) subunits but not complex III. HG reduced the expression of ATP5B as compared to control islets (p<0.05). Losartan up-regulated this effect (p<0.01). The amounts of SERCA2b protein, the predominant SERCA isoform in islets with the highest Ca<sup>2+</sup> affinity, were decreased with high glucose (p<0.01). Losartan partially reversed this effect. [Ca<sup>2+</sup>]<sub>i</sub> and [Ca<sup>2+</sup>]<sub>ER</sub> contents were decreased in HG condition (0.39 $\pm$ 0.01 vs 0.52 $\pm$ 0.02; p<0.001; 0.14 $\pm$ 0.05 vs 0.47 $\pm$ 0.26; p<0.0001, respectively). Losartan rescued [Ca<sup>2+</sup>]<sub>i</sub> content (0.45 $\pm$ 0.02; N.S. vs controls), and partially [Ca<sup>2+</sup>]<sub>ER</sub> content (0.25 $\pm$ 0.04;p<0.01). AT1R activation stimulates PLC, a major calcium signalling pathway. Acute addition of U-73122 to HG had no effect on [Ca<sup>2+</sup>]<sub>i</sub> concentrations but restored [Ca<sup>2+</sup>]<sub>ER</sub> content (0.44 $\pm$ 0.05; N.S. vs controls). In Min6B1 cells, HG increased the ER intracellular Ca<sup>2+</sup>-releasing channel IP3R2 protein expression (p<0.02 vs controls) and chronic exposure to U-73122 reversed this effect as Losartan.

**Conclusion:** Chronic HG exposure of human islets induces oxidative stress by UCP2 activation, leading to ROS production. HG induced SERCA2b dysregulation and reduction in calcium content. Losartan prevents these deleterious effects by maintaining a robust pool of reticular Ca<sup>2+</sup>, which has a key role in beta cell function as insulin secretion and ER health. The PLC/IP3R2 calcium pathway is involved in this AT1R-blocker protective effect.

Supported by: INSERM/UCBL

533

### Pancreatic beta cell failure mediated by mTORC1 hyperactivity and autophagic impairment

A. Bartolome<sup>1,2</sup>, M. Kimura-Koyanagi<sup>1</sup>, S. Shimizu<sup>1</sup>, A. Kanno<sup>1</sup>, C. Guillen<sup>2</sup>, M. Benito<sup>2</sup>, S.-I. Asahara<sup>1</sup>, Y. Kido<sup>1</sup><sup>1</sup>Health Sciences, Kobe University, Kobe, Japan, <sup>2</sup>Biochemistry and Molecular Biology, Universidad Complutense, Madrid, Spain.

**Background and aims:** Hyperactivation of the mammalian target of rapamycin complex 1 (mTORC1) in  $\beta$  cells is usually found in progression to type 2 diabetes. Although it has an essential role in  $\beta$  cell compensatory mechanisms, mTORC1 negatively regulates autophagy, a cytoprotective process. mTORC1 hyperactivity is also a well-described cause of endoplasmic reticulum stress and insulin resistance. We focused on the role that mTORC1 hyperactivation might be having on  $\beta$  cell failure.

**Materials and methods:** Using a mouse model with  $\beta$  cell specific deletion of TSC2 ( $\beta$ TSC2KO) and consequently mTORC1 hyperactivation, processes such as autophagy, mitophagy and ER stress were analyzed. Pancreatic sections or isolated islets were used for western blot, immunohistochemistry and laser scanning confocal microscopy of single intact islets for tomographic imaging of whole islets or single  $\beta$  cells within the islet.

**Results:** mTORC1 hyperactivation drives an increase in  $\beta$  cell mass mediated by cell hypertrophy. But from 40 weeks of age  $\beta$  cell mass decline and hyperglycemia appears, as we described before using  $\beta$ TSC2KO. We found FoxO1 nuclear accumulation through life, together with several apoptosis and ER-stress markers induced in islets of older  $\beta$ TSC2KO, as well as accumulation of p62/SQSTM1 and impaired ex vivo autophagic response of islets to diverse



stimuli. Mitochondrial mass is also increased on  $\beta$  cells of  $\beta$ TSC2KO mice, but interestingly several mitochondria with collapsed mitochondrial potential and positive p62/SQSTM1 staining accumulated in older islets, meaning that mitophagy together with autophagy is defective under mTORC1 hyperactivation in  $\beta$  cells.

**Conclusion:** Here we provide the in vivo evidence of  $\beta$  cell autophagy impairment as a link between mTORC1 hyperactivation and mitochondrial dysfunction, probably contributing to  $\beta$  cell failure.

Supported by: MEXT, MEC

## 534

### 14-3-3 proteins are required for pancreatic beta cell survival and glucose homeostasis

G.E. Lim<sup>1</sup>, M. Piske<sup>1</sup>, H.S. Ramshaw<sup>2</sup>, M.A. Guthridge<sup>3</sup>, A.F. Lopez<sup>2</sup>, J.D. Johnson<sup>1</sup>;

<sup>1</sup>Cellular and Physiological Sciences, University of British Columbia,

Vancouver, Canada, <sup>2</sup>Centre for Cancer Biology, Adelaide, Australia,

<sup>3</sup>Monash University, Melbourne, Australia.

**Background and aims:** Diabetes is caused by pancreatic  $\beta$ -cell death and dysfunction, which are regulated by survival and apoptotic signaling pathways. 14-3-3 proteins are molecular adaptors that integrate signaling pathways, but their roles in  $\beta$ -cell survival and glucose homeostasis are unknown. We previously reported that insulin protects  $\beta$ -cells by Raf1-mediated inhibition of Bad, both of which bind to 14-3-3 proteins. Furthermore, 14-3-3 proteins interact with effectors in insulin signaling pathways that mediate cell survival and glucose uptake. Therefore, we hypothesized that 14-3-3 proteins are crucial for  $\beta$ -cell survival and glucose homeostasis.

**Materials and methods:** To test the role of 14-3-3 proteins in  $\beta$ -cell fate and function, protein over-expression and siRNA were used in MIN6  $\beta$ -cells and mouse islets. Quantitative PCR and fluorescent microscopy were used to detect mRNA, protein localization, cell death, and proliferation. Insulin secretion was measured by RIA. Protein interactions and phosphorylation were detected by biochemical techniques. Systemic 14-3-3 $\zeta$  knockout (KO) mice were subjected to intraperitoneal glucose (2 g/kg) and insulin (0.75 U/kg) tolerance tests.

**Results:** All 14-3-3 isoforms are present in primary mouse islets with distinct patterns of distribution. Expression of difopein, a pan-14-3-3 inhibitor, promoted  $\beta$ -cell apoptosis, as observed by a 1.5-fold elevation ( $p<0.05$ ) in cleaved caspase-3 levels and increased localization of pro-apoptotic Bad in mitochondrial fractions. Difopein expression also attenuated insulin release from MIN6 cells and mouse islets. Isoform-specific knockdown revealed the involvement of the  $\zeta$ ,  $\theta$ , and  $\gamma$  isoforms in  $\beta$ -cell survival and proliferation. No effects on insulin release were observed after knockdown of each isoform, but up to 1.8-fold ( $p<0.05$ ) increases in insulin content were observed among isoforms. Over-expression of 14-3-3 $\zeta$  promoted survival following pro-inflammatory cytokine or thapsigargin exposure, in addition to decreased Bad and Bax in mitochondrial fractions. Exposure to insulin or GLP-1 increased binding of Bad and Bax with 14-3-3 $\zeta$ , suggesting their cytosolic sequestration. The inhibitory role of 14-3-3 $\zeta$  on Bad was further tested with 14-3-3 $\zeta$  binding-deficient Bad mutants, which increased cell death by up to 4-fold ( $p<0.05$ ) and promoted their mitochondrial localization. Over-expression of 14-3-3 $\zeta$  also significantly reduced the induction of pro-apoptotic DP5/Hrk and PUMA by cytokines. To examine its role in glucose homeostasis, 14-3-3 $\zeta$  KO were characterized. At 8 weeks of age, KO mice were glucose intolerant with normal insulin sensitivity, which deteriorated at 26 weeks of age. KO mice weighed 1.4-fold ( $p<0.05$ ) more than control mice. Random-fed plasma insulin and triglyceride levels were increased by 2.1- and 1.4-fold ( $p<0.05$ ), respectively, in KO mice.

**Conclusion:** The present study represents the first systematic analysis of the 14-3-3 protein family in  $\beta$ -cells. 14-3-3 $\zeta$  exerts its protective effects by affecting the localization and expression of apoptotic Bcl-2 proteins. Furthermore, loss of 14-3-3 $\zeta$  is associated with glucose intolerance and insulin resistance. Alterations in the expression of 14-3-3 proteins may represent a novel therapeutic approach to modulate insulin signaling and promote beta cell survival and function.

Supported by: CIHR, MSFHR, and JDRF

## 535

### Serine/threonine protein phosphatase 5 is involved in pancreatic beta cell apoptosis induced by dexamethasone

L. Fransson, Å. Sjöholm, H. Orsäter;

Department of Clinical Science and Education, Södersjukhuset, Unit for Diabetes Research, Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** Patients subjected to long term treatment with glucocorticoids (GCs) are at risk of developing steroid induced diabetes. Insulin production, insulin secretion and  $\beta$ -cell viability are all parameters that are negatively affected by GC treatment. GCs exert their action by binding to the GC receptor (GR). However, several GR interacting proteins modulate GR activity in response to hormone binding. Non active GR in the cytosol is bound to an Hsp90 complex associated with serine/threonine protein phosphatase 5 (PP5; *Ppp5c*) and it has been proposed that PP5 can affect the phosphorylation status of the GR, and thus affect GR signalling. It has also been shown in lymphoid cells that activation of p38 MAPK is induced in response to GC exposure and that serine 211 of the human GR is a target for p38 MAPK. Thus PP5 and p38 MAPK may reciprocally control the phosphorylation status of the GR. This study was undertaken to monitor activation of p38 MAPK in response to a GC challenge and to investigate the role of PP5 for GC activity in pancreatic  $\beta$ -cells.

**Materials and methods:** MIN6 cells were exposed to 100 nmol/l of the GC dexamethasone (dexa) with or without 1  $\mu$ mol/l of the GR antagonist RU486 for 30 h or 100 nmol/l dexa with or without 10  $\mu$ mol/l of the p38 MAPK inhibitor SB203580. Apoptosis was evaluated by measuring cytoplasmic DNA-histone complexes with ELISA. Other MIN6 cells were transfected with an siRNA targeting PP5 or a non targeting siRNA and cultured with or without 100 nmol/l dexa for 30 h. Pancreatic islets from *Ppp5c*<sup>-/-</sup> mice and *Ppp5c*<sup>+/-</sup> littermates were isolated and exposed to 200 nmol/l dexa for 48 h. Proteins were extracted and levels of cleaved caspase 3 (CC3), as a measure of apoptosis, and phosphorylation of p38 MAPK were determined with Western blotting. Data are presented as mean $\pm$ SEM. Student's *t*-test or one way ANOVA was used for statistical comparison.

**Results:** Dexa significantly induced apoptosis in MIN6 cells, both as measured with ELISA and with levels of CC3. Dexa also significantly increased phosphorylation levels of p38 MAPK. These effects were mediated via the GR since it could be fully prevented by RU486. The apoptotic effect of dexa could also be partly prevented by the p38 MAPK antagonist. MIN6 cells with reduced levels of PP5 (mean PP5 down regulation 70%) were more sensitive to the apoptotic effect of dexa, both as measured with ELISA (neg. siRNA 185.7 $\pm$ 4.6% and PP5 siRNA 277.5 $\pm$ 14.3%  $p<0.05$ ) and levels of CC3 (neg. siRNA 734.0 $\pm$ 110.4% and PP5 siRNA 1040.0 $\pm$ 160.4%  $p<0.05$ ) compared to control cells. Also phosphorylation of p38 MAPK was induced by dexa to a greater extent in cells with reduced levels of PP5 (neg. siRNA 368.9 $\pm$ 70.71% and PP5 siRNA 523.6 $\pm$ 131.9%) compared to untreated cells. The same trend was seen in isolated islets from *Ppp5c*<sup>-/-</sup> mice, they were more sensitive to dexa induced apoptosis as measured with levels of CC3 (*Ppp5c*<sup>+/-</sup> 136.0 $\pm$ 32.7% and *Ppp5c*<sup>-/-</sup> 233.5 $\pm$ 57.1%) and they showed increased p38 MAPK phosphorylation (*Ppp5c*<sup>+/-</sup> 104.8 $\pm$ 7.9% and *Ppp5c*<sup>-/-</sup> 146.0 $\pm$ 13.7%) compared to control islets.

**Conclusion:** Our data show that MIN6 cells with reduced levels of PP5 and isolated pancreatic islets lacking PP5 are more sensitive to the cytotoxic effects of dexa, as seen by increased apoptosis. This increased sensitivity is mediated via a mechanism that involves enhanced activation of p38 MAPK. Thus PP5 is a regulator of GC action in pancreatic  $\beta$ -cells.

Supported by: SLS, OE Byggmästare Foundation, DRWF, Eva and Oscar Ahréns Foundation, SSMF

## 536

### Paracrine signalling loops in adult pancreatic islets: SLIT-ROBO signalling modulates adult beta cell survival

Y.H.C. Yang, J.D. Johnson;

Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada.

**Background and aims:** Adult pancreatic islets contain multiple cell types that secrete well characterized hormones. Although it is becoming increasingly apparent that islets release and respond to more secreted factors than previously thought, systematic analyses are lacking. We have previously compiled a list of secreted factors and receptors that are expressed in adult mouse or human islets and discovered novel paracrine signaling loops within the islets,

including axon guidance factors important for neuronal development. The aim of the current study was to examine the pro-survival roles of SLIT-ROBO signaling in beta-cells. In addition to axon guidance, SLIT-ROBO signaling has also been implicated in the regulation of cell migration, angiogenesis and cell death during development of lung, kidney, liver and mammary glands.

**Materials and methods:** The expression of SLIT and ROBO family members were analyzed by quantitative RT-PCR, immunostaining, and western blotting. Mouse islet and MIN6 cells were treated with 10 nmol/L of SLIT-1 or SLIT-2 recombinant proteins in 5, 15, or 20 mmol/L glucose containing media. ER (endoplasmic reticulum) stress induced cell death was assessed by incorporation of propidium iodide, western blotting for members of the apoptosis pathway and qRT-PCR of ER stress related genes. Changes in cytosolic and ER luminal calcium levels were assessed by ratiometric imaging of cells loaded with Fura-2AM and transfected with FRET-based DIER camaleon, respectively.

**Results:** Immunostaining of adult islets revealed SLIT-2 expression exclusively in beta-cells, while ROBO1 and ROBO2 receptors were detected in beta-cells and alpha-cells. Exposing beta-cells to recombinant SLIT-2 under hyperglycemic conditions decreased thapsigargin induced cell death and down-regulated cleaved caspase-3 and CHOP protein levels. This was correlated with the decrease in Ask1-p38MAPK pathway activation and Xbp1 expression. SLITs are known to trigger repulsive axon guidance signals by regulating intracellular  $\text{Ca}^{2+}$  levels. In beta-cells, recombinant SLIT-1 and SLIT-2 treatments could deplete ER  $\text{Ca}^{2+}$  levels and increase cytosolic  $\text{Ca}^{2+}$  oscillations under 15 mmol/L glucose conditions, suggesting that  $\text{Ca}^{2+}$  is involved in SLIT induced downstream signaling.

**Conclusion:** Together, our results point to a possible context-dependent pro-survival role for SLIT-ROBO signaling in the adult pancreatic beta-cells. Since diabetes results from a deficiency in functional beta-cell mass, these studies could potentially contribute to the development of novel therapies to improve beta-cell survival.

Supported by: JDRF, SCN, MSFHR, CIHR, NSERC

## 537

### Effects of pancreatic stellate cell on islet amyloid deposition

J.M. Yin<sup>1,2</sup>, Z.L. Sun<sup>1,2</sup>, F.F. Li<sup>1,2</sup>, Q. Zhai<sup>1,2</sup>

<sup>1</sup>Department of Endocrinology, Zhongda Hospital, <sup>2</sup>Institute of Diabetes, Medical School, Southeast University, Nanjing, China.

**Background and aims:** Islet amyloid deposition is a pathological hallmark of type 2 diabetes, which mainly consists of islet amyloid polypeptide, also called amylin. However, its pathogenesis has not been fully understood. Activated pancreatic stellate cell (PSC) plays a vital role in pancreatic fibrosis and increasing evidences show that it also has additional effects on islets. Therefore, we intend to explore whether activated PSC could affect the expression of amylin.

**Materials and methods:** 1. To establish Ins-1/PSC co-culture system, PSCs were isolated from male Sprague-Dawley rats, Ins-1 cells were incubated in the media containing 5.5 or 16.7 mM glucose, cultured with or without activated PSCs. The supernatants of each group were collected for amylin concentration measurement by ELISA after 1, 2, and 6h. Ins-1 cells were harvested for amylin mRNA expression detection by Real Time-PCR after 6, 12, and 24h. 2. To establish islet/PSC co-culture system, islets and PSCs were isolated from male Sprague-Dawley rats, after overnight recovery, islets were transferred to culture media containing 5.5 or 16.7 mM glucose, cultured with or without activated PSCs. After 6, 12, and 24h, the supernatants of each group were collected for amylin concentration measurement by ELISA. Islets were harvested for amylin mRNA expression detection by Real Time-PCR and amylin protein content detection by ELISA.

**Results:** 1. At the end of each incubation period, the secretion and gene expression of amylin of Ins-1 cells cultured in the media containing 16.7 mM glucose markedly increased compared with those cultured in 5.5mM glucose ( $p<0.05$ ). The secretion of amylin increased by the presence of PSCs, whether cultured in 5.5 or 16.7 mM glucose ( $p<0.05$ ). The gene expression of amylin increased by the presence of PSCs when cultured in 16.7 mM glucose ( $p<0.05$ ). 2. At the end of each incubation period, the secretion of amylin of islets co-cultured with PSCs were significantly higher than those cultured without PSCs, whether treated with 16.7 mM glucose or 5.5mM glucose ( $p<0.05$ ). The amylin protein content and gene expression in the islets were in accordance with the secretion measurement ( $p<0.05$ ).

**Conclusions:** 1. PSC may promote the secretion of amylin in Ins-1 cells. 2. High glucose could promote the secretion and expression of amylin in Ins-1 cells, and PSC may enhance high glucose inducing amylin secretion and ex-

pression. 3. PSC may promote the secretion and expression of amylin in islets. 4. High glucose could promote the secretion and expression of amylin, and PSC may enhance high glucose inducing amylin secretion and expression.

	secretion of amylin(ng/L)		amylin protein content(ng/L)		amylin gene expression	
	islet only	islet cultured with PSC	islet only	islet cultured with PSC	islet only	islet cultured with PSC
5.5mM glucose						
6h	154.94±11.22	197.11±12.07	173.81±13.18	217.64±15.33	1.10±0.031	1.60±0.041
12h	217.13±13.94	277.63±15.03	192.12±14.32	227.13±17.26	2.38±0.052	2.77±0.071
24h	221.25±12.13	257.89±13.74	201.65±15.71	232.07±19.34	3.38±0.079	3.76±0.092
16.7mM glucose						
6h	196.05±12.25	227.02±11.17	202.72±18.48	224.20±21.69	2.05±0.052	2.58±0.043
12h	265.81±15.87	390.62±18.93	304.76±19.16	398.10±25.18	2.79±0.051	4.56±0.097
24h	318.76±11.56	353.67±10.15	310.13±20.39	336.499±20.43	3.70±0.063	4.19±0.092

Supported by: NSFC 30971399

## 538

### UCH-L1 deficiency in mice exacerbates human islet amyloid polypeptide toxicity in beta cells: evidence of cross-talk between the ubiquitin/proteasome system and autophagy

S. Costes, T. Gurlo, J.F. Rivera, P.C. Butler;

Larry Hillblom Islet Research Center, UCLA, Los Angeles, USA.

**Background and aims:** Type 2 diabetes (T2DM) is characterized by a deficit in beta-cell mass due to beta-cell apoptosis induced, at least in part, by the toxic forms of misfolded islet amyloid polypeptide (IAPP). We recently reported that increased expression of human-IAPP (h-IAPP) disrupts the ubiquitin/proteasome pathway in beta-cells of individuals with T2DM, as demonstrated by the accumulation of polyubiquitinated proteins associated with the deficiency in the deubiquitinating enzyme UCH-L1 (Ubiquitin C-terminal hydrolase-L1). Using h-IAPP transgenic rodent models, we also recently reported that expression of amyloidogenic h-IAPP in beta-cells compromises the second pathway of protein degradation known as autophagy. In this study, we used mouse genetics to elucidate the potential synergistic effect of UCH-L1 deficiency and excess of oligomerization prone h-IAPP in diabetes progression, and we further investigated whether UCH-L1 deficiency could exacerbate autophagy defects in this mouse model.

**Materials and methods:** Mice overexpressing h-IAPP in UCH-L1-deficient beta-cells (UCH-L1<sup>-/-</sup>, h-IAPP-Tg) were obtained by crossbreeding h-IAPP transgenic mice and the nm3419 mice harboring a deletion mutation in the *Uchl1* gene. Wild type, UCH-L1<sup>+/-</sup> and h-IAPP-Tg mice were used as controls. The study has been carried out along the "Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985) and according to the national law. UCH-L1 deletion was confirmed by genomic PCR, immunoblotting and measurement of UCH-L1 activity in isolated mouse islets. Fasting blood glucose and body weight were measured weekly starting from 5 weeks. Pancreases were collected from 7-8 week-old mice to evaluate islet morphology, beta-cell mass and beta-cell death (TUNEL) by immunofluorescence. Apoptosis in islets was also evaluated by measuring levels of cleaved caspase-3 by immunoblotting. Autophagy was assessed by immunoblotting and immunofluorescence for LC3-II, a marker of autophagosome number, and for p62, an ubiquitin-binding protein, which interacts with LC3-II, allowing lysosomal degradation of polyubiquitinated proteins and itself.

**Results:** UCH-L1<sup>-/-</sup>, h-IAPP-Tg mice became hyperglycemic at 7 weeks (average glycemia 139 mg/dL,  $p<0.05$  vs h-IAPP-Tg mice), and thereafter exhibited severe diabetes, while control groups remained normoglycemic. Beta-cell mass declined in UCH-L1<sup>-/-</sup>, h-IAPP-Tg mice, showing 61% reduction at 7 weeks of age ( $p<0.001$  vs h-IAPP-Tg mice), accompanied by remodeling of islet architecture and increased beta-cell apoptosis. Analysis of autophagy in UCH-L1<sup>-/-</sup>, h-IAPP-Tg mice revealed an increase in the number of autophagosomes (1.6 fold increase in LC3-II levels,  $p<0.01$  vs h-IAPP-Tg mice) and a major increase in the number and size of cytoplasmic inclusions positive for p62 and ubiquitin (28.8% of beta-cells positive for inclusions vs 3.51% in h-IAPP-Tg mice,  $p<0.001$ ).

**Conclusion:** These data suggest that UCH-L1 deficiency exacerbated h-IAPP-induced beta-cell death caused at least in part by the amplified altera-

tion of the lysosome-dependant degradation. This study reveals a previously unrecognized role for UCH-L1 in the regulation of autophagy in beta-cells exposed to misfolded h-IAPP.

Supported by: NIH (DK059579) and LLHF (2008-D-027-FEL)

## 539

### The role of chaperones in the ER stress response induced by human IAPP overexpression

L. Cadavez<sup>1,2</sup>, P. Casini<sup>1,2</sup>, M. Soty<sup>1,2</sup>, M. Visa<sup>1,2</sup>, R. Gasa<sup>1,2</sup>, V. Petegnief<sup>3</sup>, A. Novials<sup>1,2</sup>;

<sup>1</sup>Diabetes and Obesity Laboratory, IDIBAPS, Hospital Clinic, <sup>2</sup>Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), <sup>3</sup>Department of Brain Ischemia and Neurodegeneration, CSIC, Barcelona, Spain.

**Background and aims:** Human islet amyloid polypeptide (hIAPP) has been found as a principal constituent of islet amyloid deposits. The process of hIAPP aggregation is also a common mechanism with neurodegenerative diseases in which proteins adopt non-native conformation. In many cases, misfolding is a natural consequence of protein biogenesis, but in T2D, it may contribute to  $\beta$ -cell apoptosis. In addition, accumulation of misfolding proteins perturbs the endoplasmic reticulum (ER) homeostasis activating the unfolded protein response (UPR). Molecular chaperones have been described to be important for regulation of ER signaling in response to ER stress, among others. The aim of the present work is to investigate the UPR in a cellular model of hIAPP overexpression.

**Materials and methods:** Rat pancreatic  $\beta$ -cell line INS1E was stably transfected with hIAPP plasmid (INS1E\_hIAPP cells) under the cytomegalovirus (CMV) promoter. To evaluate ER stress response, INS1E\_hIAPP cells were culture with pharmacological (0.5  $\mu$ M thapsigargin) and non-pharmacological (25 mM glucose and 400  $\mu$ M palmitic acid) ER stress inducers in a time-dependent manner. Liposome-mediated transient BiP/GRP78 overexpression was performed in INS1E\_hIAPP cells. To mimic the plausible protective role of endogenous chaperone BiP/GRP78 during ER stress, INS1E\_hIAPP cells were pre-treated with the chemical chaperone taurine-conjugated ursodeoxycholic acid (TUDCA). Gene expression and protein levels of ER stress markers (CHOP, ATF3 and spliced XBP1) and BiP/GRP78 were analysed by real-time RT-PCR and western blot respectively.

**Results:** INS1E\_hIAPP cells cultured at 11 mM glucose did not show changes in the ER stress markers compared to INS1E control cells. Both cells were exposed to 0.5  $\mu$ M thapsigargin, an inducer of ER stress, for 8h. Interestingly, the expression of ER stress genes was higher in INS1E\_hIAPP (CHOP 11.2-fold, ATF3 42.3-fold and spliced XBP 8.3-fold) over untreated cells ( $p < 0.001$ ) compared to INS1E cells (CHOP 5.9-fold, ATF3 18.5-fold and spliced XBP1 4-fold) over untreated cells ( $p < 0.001$ ). At 24h, thapsigargin-treated INS1E\_hIAPP cells showed an increase in CHOP and ATF3 protein levels in relation to INS1E cells. INS1E\_hIAPP cultured at 25 mM glucose did not show changes in gene expression compared to basal glucose (11 mM). The addition of 400  $\mu$ M palmitic acid to 25 mM glucose induced an increase of ATF3 and spliced XBP1 mRNA expression levels of 4.2-fold, and 3.5-fold, respectively compared to basal conditions. At 24h INS1E\_hIAPP showed an increase in BiP protein levels compared to INS1E cells. Overexpression of BiP in INS1E\_hIAPP cells cultured with high glucose and palmitic acid did not modify the expression profile of ER stress markers. However, cells exposed to high glucose and palmitic acid treated with 200  $\mu$ M of TUDCA, showed a small but statistically decrease of ATF3 and spliced XBP1 genes (13%,  $p < 0.05$  and 18%,  $p < 0.05$  respectively).

**Conclusion:** Overexpression of hIAPP is sensed by ER and triggers the activation of UPR and enhances chaperone expression. Thereby, TUDCA seems to ameliorate ER stress, suggesting that improving chaperone capacity can be important to the folding of hIAPP.

Supported by: FIS (PI08/0088 and PI11/00679)

## 540

### Role of the $\beta$ -secretase BACE2 in the mechanism of hIAPP-induced beta cell dysfunction

G. Alcarraz-Vizán<sup>1,2</sup>, P. Casini<sup>1,2</sup>, L. Cadavez<sup>1,2</sup>, M. Visa<sup>1,2</sup>, J.M. Servitja<sup>1,2</sup>, A. Novials<sup>1,2</sup>;

<sup>1</sup>Diabetes & Obesity Laboratory, IDIBAPS, Hospital Clinic, <sup>2</sup>Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), Barcelona, Spain.

**Background and aims:** BACE2 ( $\beta$ -site APP-cleaving enzyme 2) is a  $\beta$ -protease that has been found in the brain, where it plays a role in the development of Alzheimer's disease (AD). It has also been localized in the pancreas, although its biological function is not fully known. A recent report demonstrated that BACE2-KO mice showed improved control of glucose homeostasis. Moreover, amyloidogenic diseases, including AD and type 2 diabetes (T2D), have been reported to share the accumulation of abnormally folded and insoluble proteins within or around cells that interfere with cell function. In the case of T2D, amylin (IAPP) deposits have been shown to be a key feature of the disease, although the mechanism of IAPP aggregation is poorly understood. In this framework, we have found that human IAPP (hIAPP) overexpression specifically induced BACE2 upregulation, therefore the aim of the present study was to investigate the implication of the  $\beta$ -secretase BACE2 in the  $\beta$ -cell alterations induced by hIAPP overexpression.

**Materials and methods:** The rat pancreatic  $\beta$ -cell line INS1E was stably transfected with hIAPP under the control of CMV promoter (INS1E-hIAPP cells). BACE2 expression was downregulated in INS1E-hIAPP using siBACE2. A liposome-mediated transient BACE2 overexpression was performed in INS1E cells. The enzymatic activity of BACE2 was detected in protein extracts with a fluorometric  $\beta$ -secretase proteolytic activity assay. The ability to secrete insulin in response to glucose (GSIS), as well as insulin content, was quantified with an ELISA kit. Proliferation was analyzed by BrdU assay, and mitochondrial metabolism measured as ROS production.

**Results:** hIAPP-INS1E cells presented a 4.5-fold increase of BACE2 expression with respect to INS1E cells ( $p < 0.05$ ). This increase was not observed in INS1E cells overexpressing rat IAPP. Insulin secretory response to glucose was 30% lower in hIAPP-INS1E cells than in INS1E cells ( $p < 0.05$ ). INS1E cells transfected with a transient overexpression of BACE2 showed a 1.3-fold increase in BACE2 activity ( $p < 0.05$ ). These cells presented a 3-fold increase in ROS production ( $p < 0.05$ ), as well as a 40% decrease in proliferation ( $p < 0.05$ ). Insulin secretory response to glucose in INS1E cells overexpressing BACE2 was 25% lower ( $p < 0.05$ ) than in control cells. When endogenous BACE2 was silenced in hIAPP-INS1E cells, there was a significant improvement in GSIS (3-fold increase in insulin secretory response stimulated by glucose with respect to hIAPP-INS1E cells,  $p < 0.05$ ), indicating the significant effect of BACE2 expression on  $\beta$ -cell function.

**Conclusion:** BACE2 levels are increased in  $\beta$ -cell dysfunction induced by hIAPP overexpression, and its downregulation improves insulin secretory response in rat pancreatic  $\beta$ -cells.

Supported by: FIS (PI08/0088 and PI11/00679)



## PS 032 In vitro insulin action

541

### Fibroblast growth factor-21 (FGF-21) synergizes with insulin in human adipose stem cell-derived (hASC) adipocytes

M.B. Brenner, D.V. Lee, B. Bernardo, S. Talukdar;  
CVMED, Pfizer, Cambridge, USA.

Over the past years FGF21, a member of the FGF19 subfamily, has evolved as a major metabolic regulator with beneficial effects on insulin sensitivity and glucose tolerance in rodents and other species. So far, in-vitro studies studying the effects of FGF-21 on insulin sensitivity have mostly been limited to observations in rodent cells after chronic treatment with FGF-21. Our study focuses on the acute effects of FGF-21 on insulin signaling using a human model of adipocytes, with the aim of providing insights on the crosstalk between FGF-21 and insulin signaling. Human adipose stem cell-derived (hASC) adipocytes were acutely treated with FGF-21 alone, insulin alone, or in combination. Insulin signaling under these conditions was characterized by measuring [<sup>14</sup>C]-2DG uptake, tyrosine phosphorylation of insulin receptor (InsR), insulin receptor substrate-1 (IRS-1), and serine 473 phosphorylation of Akt. FGF-21 alone caused a modest increase of glucose uptake, but acute treatment of FGF-21 in combination with insulin had a synergistic effect on glucose uptake in hASC-adipocytes. The presence of FGF-21 also effectively lowered the insulin concentration required to achieve the same level of glucose uptake as in the absence of FGF-21 by 10-fold. This acute effect of FGF-21 on insulin signaling was not due to InsR, IGF-1R, or IRS-1 activation. We do observe a substantial increase in basal S473-Akt phosphorylation by FGF-21 alone, in contrast to the minimal shift in basal glucose uptake. We conclude that acute co-treatment of hASC-adipocytes with FGF-21 and insulin synergistically improves maximal glucose uptake, and the mechanism of action lies downstream of Akt or outside the canonical insulin signaling pathway.

542

### IRS (insulin receptor substrate) -2 protein stability is increased by PKA-dependent 14-3-3 binding

S.S. Neukamm<sup>1,2</sup>, J. Ott<sup>3</sup>, S. Dammeier<sup>3</sup>, R. Lehmann<sup>1,2</sup>, H.-U. Haering<sup>1,2</sup>, E.D. Schleicher<sup>1,2</sup>, C. Weigert<sup>1,2</sup>;

<sup>1</sup>Internal Medicine IV, Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry, University Hospital Tuebingen, <sup>2</sup>Paul Langerhans Institute, Member of the German Diabetes Centre, Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Centre Munich at the University of Tuebingen, <sup>3</sup>Institute for Ophthalmic Research, University of Tuebingen, Germany.

**Background and aims:** IRS-1 and -2 as intermediate docking platforms transduce the insulin/IGF-1 signal to intracellular effector molecules that regulate glucose and lipid metabolism. Binding of 14-3-3 proteins can modify the activity of the binding partner, influence trafficking or obscure other nearby motifs for other interaction partners. 14-3-3 proteins bind their target protein via phosphorylated serine/threonine residues located within distinct motifs. Additionally, IRS-2 is regulated on the mRNA and protein level. Insulin suppresses IRS-2 mRNA expression, whereas glucagon promotes IRS-2 mRNA expression. Here we studied the regulated binding of 14-3-3 to IRS-2 upon PKA (protein kinase A) activation, determined the residues that mediate this interaction and the physiological consequences thereof.

**Materials and methods:** Fragments spanning different regions of the IRS-2 molecule and mass spectrometry were used to narrow down the 14-3-3 binding region on IRS-2 and potential PKA phosphorylation sites herein. Loss of function mutants and GST (glutathion S-transferase) and co-immunoprecipitation assays were applied to confirm the responsible residues for PKA-dependent 14-3-3 binding. Flp-In HEK293 cells stably expressing IRS-2 and primary hepatocytes were employed to investigate the potential biological importance of this interaction.

**Results:** Activation of PKA by forskolin increased 14-3-3 binding to IRS-2, whereas inhibition of PKA activity by the compound H89 blocked 14-3-3 binding to IRS-2 in transiently transfected HEK293 and rat hepatoma (Fao) cells. The area behind amino acid 952 on IRS-2 could be identified as 14-3-3 binding region and mass spectrometric analyses revealed serine 1137 and serine 1138 as potential PKA phosphorylation sites. This was tested by application of an antibody that recognizes potential PKA phosphorylation sites.

GST pulldown and co-immunoprecipitation assays confirmed serine 1137 and serine 1138 to be part of 14-3-3 binding to IRS-2. Flp-In HEK293 cells stably expressing IRS-2 showed a reduction in IRS-2 content of 90 % ± 3 % (n=8) after 3 and 6 hours of cycloheximide treatment. Contrary, cells that were treated in combination with forskolin showed only a reduction in IRS-2 protein content of about 30 % ± 10 % (n=8) after 3 and 6 hours of treatment, thus IRS-2 protein showed increased stability.

**Conclusion:** We present a novel regulatory mechanism of IRS-2 protein stability - the PKA-dependent 14-3-3 binding to IRS-2.

*Supported by: DFG International Graduate School GRK 1302/1 and 1302/2; BMBF for DZD e.V.*

## PS 032 In vitro insulin action

543

### Knockdown of PRAS40 promotes proteasomal degradation of IRS1 and insulin resistance in human skeletal muscle cells

C. Wiza, D. Herzfeld de Wiza, S. Lehr, D.M. Ouwens;

Institute of Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Duesseldorf, Germany.

**Background and aims:** Insulin resistance is a hallmark of type 2 diabetes and characterized by alterations in Akt- and mammalian target of rapamycin complex 1 (mTORC1) signaling. In rodent models of insulin resistance, hyperactivation of the mTORC1/p70S6K-axis is linked to inhibition or even degradation of the insulin receptor substrate 1 (IRS1). The proline-rich Akt substrate of 40kDa (PRAS40) is a component of the mammalian target of rapamycin-complex 1 (mTORC1) and one the most prominent Akt substrates in skeletal muscle. Studies in cultured cell lines ascribed both inhibitory and stimulatory roles to PRAS40 in the regulation of mTORC1 activity and insulin action. Genetic evidence from *Drosophila* indicated that the regulation of mTORC1 activity is tissue-specific and dependent on post-translational modification of PRAS40. This study aimed to detail the function of PRAS40 in insulin action in primary human skeletal muscle cells (hSkMC) under normal conditions and following monocyte chemo-attractant protein 1 (MCP1) induced insulin resistance.

**Materials and methods:** Primary hSkMC from different donors were differentiated *in vitro* for 7 days. On day 3 of differentiation, cells were transfected with either a non-target siRNA or specific PRAS40-siRNAs. On day 6 of differentiation, myocytes were incubated with 2 ng/ml MCP1 (24h) prior to stimulation with 100 nM insulin for 10 min. For inhibition of the proteasome, PRAS40 knockdown (PRAS40 KD) and control cells were treated with 3 μM MG-132 for 24h. Alterations in the phosphorylation and protein expression of targets of Akt- and mTORC1 signaling were analyzed by Western blotting, real-time PCR or bead-based multiplex assays.

**Results:** Knockdown of PRAS40 in hSkMC decreases insulin-mediated phosphorylation of Akt by 50% ( $P<0.05$ ) and abrogated insulin action downstream of Akt, as illustrated by diminished insulin-stimulated phosphorylations of glycogen synthase kinase 3 (40%) and tuberous sclerosis complex 2 (32%) (both  $P<0.05$ ). Furthermore, in the absence of changes in Glut4 protein expression, insulin-stimulated glucose uptake was reduced by 20% in PRAS40KD versus non-target myocytes ( $P<0.05$ ). Finally, PRAS40-KD affected mTORC1 signaling as illustrated by inhibition of insulin-mediated phosphorylation of mTOR (30%), and its substrates p70S6K (47%) and 4E-BP1 (30%), as well as by reducing the protein expression of the newly identified mTORC1 target growth factor receptor bound protein 10 (Grb10) (35%) (all  $P<0.05$ ). Comparable results were obtained with two distinct PRAS40-siRNAs in cells from different donors. Exposing PRAS40KD-myocytes to MCP1 caused no further deterioration of insulin action. The abrogation of insulin action by PRAS40KD and MCP1 could be ascribed to a proteasome-mediated reduction in IRS1 protein levels (60% reduction after PRAS40KD and 55% after MCP1 treatment) (both  $P<0.05$ ). Accordingly, pharmacological inhibition of the proteasome by MG-132 restored IRS1-abundance and insulin sensitivity induced by both PRAS40 KD and MCP1.

**Conclusion:** Collectively, this study identifies PRAS40 as a modulator of insulin action in primary human skeletal muscle cells. In these cells, the absence of PRAS40 abrogates insulin action by promoting the proteasome-mediated degradation of IRS1 rather than by hyperactivation of the mTOR-p70S6K/Grb10 negative feedback loop.

544

### Analysis of signalling pathways stimulated by insulin, AspB10, insulin glargine and its metabolites in human colon cancer cell lines

J. Raschert, S. Welte, G. Tschanke, N. Tennagels;

Sanofi, Frankfurt, Germany.

**Background and aims:** Insulin glargine (GLA) is a long-acting insulin analogue that, in contrast to X10, did not show an increased incidence of mammary tumours in rats and mice in 2-year carcinogenicity studies. *In vivo*, GLA is rapidly and significantly metabolised into metabolite M1 that has a metabolic profile like GLA but a mitogenic profile identical to human insulin (HI). Our aim was to compare the mitogenic activity and signalling behaviour of GLA and M1 with IGF-1, HI and X10 in human colon cancer cell lines that

have different IGF-1 receptor (IGF1R)/insulin receptor (IR) and IR-A/IR-B ratios.

**Materials and methods:** The stimulation of IR and IGFR autophosphorylation and AKT and ERK1/2 phosphorylation as well as thymidine incorporation by HI and insulin analogues was investigated in the human colon cancer cell lines HCT116, DLD1, HT29, SW480, Colo205 and LoVo.

**Results:** X10 was the most potent stimulator of IR and IGFR autophosphorylation and AKT phosphorylation in all cell lines studied. No differences were observed between the signalling behaviour of M1 and HI in any of the cell lines. GLA displayed IR activity comparable with HI in all cell lines studied. IGFR phosphorylation was also comparable, except for an increase in HT29 and Colo205 cells compared with 50 nM HI (GLA: 1.7- and 1.5-fold; IGF-1: 5.1- and 3.2-fold), that was also reflected in decreased EC50 values for AKT phosphorylation in the 2 cell lines (GLA: 4- and 1.7-fold; IGF-1: 4- and 19-fold, respectively). ERK1/2 phosphorylation and radioactive thymidine incorporation were unaffected by insulin or analogue treatment in all cell lines. However, poly (ADP-ribose) polymerase (PARP) cleavage was significantly decreased, reflecting an anti-apoptotic response, in HCT116 cells exposed to X10 and IGF1 but not GLA or M1.

**Conclusion:** The data indicate that *in vitro* M1 displays an IR- and IGFR-mediated profile comparable with HI for all colon cancer cell lines investigated. GLA was also comparable to HI, except for an increase in IGFR and AKT phosphorylation in 2 cell lines. This emphasises the importance of studying the multiple steps in the action of an insulin analogue and its metabolites *in vitro* and *in vivo*.

Supported by: Sanofi

545

### Insulin affects tyrosine hydroxylase expression in neuronal PC12 cells through Hif-1alpha activation

F. Fiory<sup>1</sup>, P. Mirra<sup>1</sup>, C. Nigro<sup>1</sup>, L. Ulianich<sup>1</sup>, M. Lucariello<sup>1</sup>, F. Zatterale<sup>1</sup>, A. Leone<sup>1</sup>, P. Formisano<sup>2</sup>, F. Beguinot<sup>1,2</sup>, C. Miele<sup>1</sup>;

<sup>1</sup>IEOS, CNR, <sup>2</sup>University Federico II, Naples, Italy.

**Background and aims:** Dopamine plays a key role in regulating behavior and cognition, sleep, mood and learning. Dysfunctions of the dopamine system lead to neurodegenerative disease such as Parkinson's disease, an age-related degenerative condition causing tremor and motor impairment. It has been recently shown that streptozotocin-diabetic rats have lower catecholamines levels in the corpus striatum and immunoreactive tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamines synthesis, is decreased in nigrostriatal neurons of the genetically diabetic BB Wistar rats. Recent studies show that diabetes represents a risk factor for dementia and is associated to an increased risk of Parkinson's disease. Moreover, impairment of insulin synthesis or action is associated with neurodegenerative diseases and with a decline in cognitive function.

**Materials and methods:** TH protein and mRNA levels in PC12 cells were evaluated by Western blot and RT-PCR respectively. Chlp experiments and luciferase assays were performed to evaluate Hif-1alpha role in regulating TH expression in response to insulin.

**Results:** We have evaluated insulin ability to regulate TH levels in PC12 cells. TH mRNA levels are increased by 50% upon 1-2 h of 100 nM insulin treatment and return to basal values after 6-8 h of insulin stimulation. Prolonged incubation (16-24h) of PC12 cells with insulin induces a further gradual increase in TH mRNA expression. By contrast, TH protein levels are increased after 1 hour of insulin stimulation and are still elevated upon 24h of insulin treatment. Pharmacological inhibition of the transcription factor Hif-1alpha, a known regulator of TH expression, almost completely abolishes insulin positive effect on TH mRNA. Moreover, insulin stimulation increases the binding of Hif-1alpha and of its cofactor p300 to TH promoter and induces the reporter gene activity of a TH promoter-luciferase construct in HeLa cells. This effect is lost when cells are transfected with a dominant negative of Hif-1alpha or with a vector containing the mutagenized Hif-1alpha binding sequence.

**Conclusion:** These results suggest that insulin regulates TH mRNA expression by activating Hif-1alpha. Improvement of insulin signal transduction in brain could preserve neurotransmitters content in diabetic condition.

## 546

**Impact of exogenous follistatin on primary human myotubes**P. Plomgaard<sup>1,2</sup>, B.K. Pedersen<sup>1</sup>, P.A. Halban<sup>3</sup>, K. Bouzakri<sup>3</sup><sup>1</sup>Centre of Inflammation and Metabolism, Rigshospitalet, Copenhagen, Denmark, <sup>2</sup>Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark, <sup>3</sup>Department of Genetic Medicine and Development, Medical Centre, Geneva University, Switzerland.

**Background and aims:** Follistatin is a protein found in the circulation. It has recently been demonstrated that plasma follistatin increases rapidly after an acute bout of exercise. This finding indicates a new role for follistatin, which so far mainly has been investigated in reproduction physiology. Follistatin antagonises and neutralises several members of the TGF- $\beta$  family in the circulation. Of particular interest in relation to exercise is myostatin or GDF-8. Myostatin is produced and secreted by the skeletal muscle, where it suppresses growth and hypertrophy, and is believed to be neutralised in the circulation by follistatin. Here we investigate the effect of exogenous follistatin on human skeletal muscle cells.

**Materials and methods:** Recombinant human follistatin was used to treat human primary myotubes. Myoblasts were isolated from human abdominal skeletal muscle. These were grown into confluence and differentiated for 5 days with and without 50 ng/ml recombinant human follistatin. mRNA was measured by qRT-PCR. Glucose uptake was measured by incorporation (10 min) of 3H-2-deoxyglucose.

**Results:** Human primary myotubes differentiated in the presence of human recombinant follistatin resulted in a hypertrophic myotube when compared to the non-treated condition. mRNA was isolated from the differentiated myotubes and a higher level of MEF2A (1.6  $\pm$  0.14 fold,  $P < 0.05$ ) and MEF2C (3.8  $\pm$  1.2 fold,  $P < 0.05$ ) was found in the follistatin differentiated myotubes. Differentiation with follistatin suppressed MEF2D mRNA 1.9 fold ( $P < 0.05$ ). Interestingly GLUT1 mRNA was suppressed 1.4 fold ( $P < 0.05$ ) whereas GLUT4 was increased 1.6 fold ( $P < 0.05$ ), after differentiation with follistatin. Because follistatin changed the expression of the glucose transporters, glucose uptake was measured with 0, 10 and 100 nM insulin. Without insulin a 1.7 fold reduction in glucose uptake was observed after differentiation with follistatin. A low dose of insulin (10 nM) elicited a 1.4 fold ( $P < 0.05$ ) increase in glucose uptake only in the follistatin treated but not control myotubes, whereas at 100 nM insulin similar absolute levels of glucose uptake were seen.

**Conclusion:** These data indicate that increased exogenous follistatin during muscle differentiation results in a more insulin sensitive cellular phenotype. Furthermore, as models of follistatin over-expression within skeletal muscle have also demonstrated, exogenous follistatin can induce skeletal muscle cell hypertrophy. Taken together these data suggest that exogenous follistatin has a beneficial impact on the skeletal muscle and support the idea that follistatin could have a therapeutic potential.

*Supported by: EFSD Albert Renold Fellowship (to PP); Disere og Niels Yde Foundation*

## 547

**LY2881835: a potent, efficacious and selective GPR40 agonist stimulates insulin and GLP-1 secretion normalising glucose levels in preclinical models of type 2 diabetes**A. Reifel Miller<sup>1</sup>, A. Cox<sup>1</sup>, D. Briere<sup>1</sup>, M. Marcelo<sup>1</sup>, S. Rani S.N.<sup>2</sup>, R. Padmaja.<sup>2</sup>, K. Wilbur<sup>1</sup>, E. Osborne<sup>1</sup>, R. Zink<sup>1</sup>, D. Jett<sup>1</sup>, C. Montrose-Rafizadeh<sup>1</sup>, M. Michael<sup>1</sup>, K. Bokvist<sup>1</sup>, P. Matti<sup>2</sup>, C. Hamdouch<sup>1</sup><sup>1</sup>Endocrinology, Lilly Research Laboratories, Indianapolis, USA, <sup>2</sup>Jubilant BioSys Ltd, Bangalore, India.

**Background and aims:** GPR40, also known as free fatty acid receptor 1 (FFAR1), is located primarily on endocrine cells of the pancreas and in the gastrointestinal (GI) tract. Engagement of GPR40 by native and synthetic ligands activates the G-protein heterotrimer G $\alpha$ q/11 resulting in elevated intracellular calcium levels. In pancreatic islet beta cells, activation of this pathway results in glucose-dependent insulin secretion (GDIS). Thus, an orally available synthetic GPR40 agonist would be expected to lower glucose levels only in the setting of hyperglycemia, potentially providing an efficacious therapeutic approach for the treatment of type 2 diabetes (T2D) that avoids hypoglycemia.

**Materials and methods:** LY2881835 is a novel spiro[indene-1,4'-peperidine] identified via a medium throughput screen followed by rationale design optimization. This molecule was characterized in-vitro for GPR40 activity and selectivity plus in-vivo as a potential therapeutic option for T2D.

**Results:** LY2881835 is a potent (233nM) and efficacious (92%) GPR40 full agonist when compared with the native ligand, linoleic acid, in a calcium flux assay (FLIPR) assay using a recombinant hGPR40/HEK293 cell line. This member of a new structural class of GPR40 agonists demonstrates selectivity against PPARs and stimulates glucose dependent insulin secretion (GDIS) in the mouse insulinoma cell line Min6, and in primary islets from mice, rats and humans. When administered orally at 3 mg/kg to Sprague Dawley rats 60 minutes prior to the start of a hyperglycemic (250 mg/dl) clamp, LY2881835 produced an immediate and prolonged increase in insulin secretion and required a significant elevation (3-fold) in the glucose infusion rate thus confirming in-vivo target engagement and activation of GPR40. After four days of oral administration of LY2881835 to normal mice, potent dose dependent reductions in glucose levels ( $ED_{50} = 0.58$  mg/kg) along with significant increases in insulin secretion were seen during an ipGTT. When chronically administered to Zucker fa/fa rats, glucose levels during an OGTT were dose dependently lowered with similar  $ED_{50}$ s on days 1 and 21 indicating that desensitization of the receptor did not occur after chronic activation. Cell-based and in-vivo studies were performed to explore the ability of LY2881835 to activate GPR40 in the GI tract. An immediate and prolonged increase in GLP-1 secretion was seen when LY2881835 was administered orally to normal mice at 30 mg/kg, plus a dose dependent increase in GLP-1 secretion was seen in the mouse gut tumor cell line, STC-1.

**Conclusion:** Synthetic, orally available GPR40 agonists that simulate GDIS plus GLP-1 secretion make excellent clinical candidates for the early and aggressive treatment of T2D without the liability of hypoglycemia

## 548

**Different acetylation patterns determine the translocation behaviour of FOXO1**M.A. Osterhoff<sup>1,2</sup>, C. Bumke-Vogt<sup>2,1</sup>, S. Schiess<sup>2</sup>, V. Baehr<sup>1</sup>, D. Schmoll<sup>3</sup>, A.F.H. Pfeiffer<sup>1,2</sup><sup>1</sup>Endocrinology, Diabetes and Nutrition, Charité - University Medicine Berlin, CBF, Berlin, <sup>2</sup>Clinical Nutrition, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, <sup>3</sup>Sanofi-Aventis Germany GmbH, Frankfurt am Main, Germany.

**Background and aims:** FOXO1 is a transcription factor ubiquitously expressed in human cells which facilitates different tasks like metabolic and energy homeostasis, development, and protection to cellular stress. The proper and specific function of FOXO is mediated by a set of post-translational modifications like phosphorylation, acetylation, ubiquitinylation, and sumoylation. While it is well known that phosphorylation at ser256 is the gate-keeper for the dissociation of FOXO from DNA and, thus, for its inactivation, there is only insufficient knowledge of the function of different acetylation patterns. The aim of this study is to investigate the function of these acetylation patterns on FOXO activity and localization.

**Materials and methods:** A FOXO translocation assay has been established using FOXO1 with a C-terminal GFP-tag which is constitutively expressed in stably transfected U-2OS (human osteosarcoma cell line) or transiently transfected into HepG2 (human hepatoma) cells and detected by fluorescent microscopy in real-time. Site directed mutagenesis was used to create different constructs where lysine is mutated to arginine to mimic de-acetylated sites. Nuclear exclusion was initiated by use of 100 nmol/L insulin, nuclear import by use of 50  $\mu$ mol/L resveratrol or luteolin.

**Results:** While the nuclear exclusion of w.t. FOXO1-GFP with 100 nmol/L insulin was accomplished in approx. 10 min, a construct where lysine 245, 248 and 265 were mutated to arginine needed nearly 30 min for nuclear export under the same conditions. Interestingly, a construct where lysine 245 and 265 were mutated to arginine translocated more slowly into the cytosol compared to the w.t. FOXO1-GFP and accumulated in a peri-nuclear manner. The w.t. and mutated constructs stably re-translocated into the nucleus with resveratrol or luteolin. Mutation of lysine 274 and 294 resulted in nuclear exclusion of FOXO1-GFP after administration of 100 nmol/L insulin in a similar time-course as the w.t. FOXO1-GFP. Luteolin caused a nuclear re-import of the K274R/K294R construct but in contrast to the constructs tested before this one re-translocated into the cytosol after 20 min without adding any additional stimulus.

**Conclusion:** The experiments support the hypothesis that acetylation of FOXO1 facilitates an easier phosphorylation of FOXO1 leading to a faster dissociation of FOXO1 from DNA and faster nuclear exclusion. Constructs where several acetylation sites were mutated showed a delayed translocation. Acetylation patterns also seem to define the subcellular localization of



FOXO1. Therefore, analysis of FOXO1-acetylation pattern might be a valuable tool to determine FOXO1 function and the metabolic state of a cell.

Supported by: "Top Projects" cooperation between Charité and Sanofi-Aventis

## 549

### Cyclosporin A and tacrolimus can reduce GLUT4 at the cell surface via increased endocytosis and this leads to impaired cellular glucose uptake

M.J. Pereira<sup>1,2</sup>, J. Palming<sup>1</sup>, M. Rizell<sup>3</sup>, M. Aureliano<sup>2</sup>, E. Carvalho<sup>4</sup>, M.K. Svensson<sup>5</sup>, J.W. Eriksson<sup>1,6</sup>

<sup>1</sup>The Lundberg Laboratory for Diabetes Research, Department of Molecular and Clinical Medicine, Sahlgrenska Academy at University of Gothenburg, Sweden, <sup>2</sup>Center for Marine Sciences (CCMar), DCBB/FCT, University of Algarve, Faro, Portugal, <sup>3</sup>Department of Surgery, Sahlgrenska Academy at University of Gothenburg, Sweden, <sup>4</sup>Center of Neuroscience and Cell Biology, University of Coimbra, Portugal, <sup>5</sup>Department of Molecular and Clinical Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden, <sup>6</sup>AstraZeneca R&D, Mölndal, Sweden.

**Background and aims:** The immunosuppressive agents (IA), cyclosporine (CsA) and tacrolimus (FK), are both calcineurin inhibitors and they are associated with several side effects including hyperglycaemia and new-onset diabetes after organ transplantation. We investigated the direct effects of therapeutic concentrations of CsA and FK on glucose uptake and insulin signalling in human adipocytes and on regulation of cellular trafficking of the glucose transporter GLUT4.

**Materials and methods:** Glucose uptake and protein expression of insulin signalling proteins were measured in isolated subcutaneous adipocytes, obtained from 42 healthy volunteers, incubated in the absence and presence of CsA or FK and insulin (1000 µU/mL). Effects of IA on cellular distribution of GLUT4 in human preadipocytes differentiated into adipocytes, was evaluated by immunohistochemistry and confocal microscopy. In addition, effects of IA on endocytotic and exocytotic rates of the transporter were studied in L6 myoblasts stably expressing GLUT4 with an exofacially directed Myc-tag by an enzyme-linked immunosorbent-like assay.

**Results:** CsA and FK both had a concentration dependent inhibitory effect on basal and insulin-stimulated <sup>14</sup>C-glucose uptake in adipocytes (1nM-1µM: ~10 to 40%, p<0.05). Although the phosphorylation of IR at Tyr1146 was inhibited by CsA and FK, phosphorylation and/or protein levels of proximal insulin signalling proteins (IRS1/2, p85-PI3K, PKB, AS160, mTOR1C) and GLUT1 and 4 content were not changed upon incubation of adipocytes with IA. Furthermore, incubation of differentiated human adipocytes with CsA or FK led to a reduction of GLUT4 localized at the cell surface (by 50–80%, p<0.05). In addition, CsA and FK similarly reduced the cell surface levels of GLUT4 in L6 muscle cells, by ~20% (p<0.05), and increased the GLUT4 endocytosis rate, by up to 30% (p<0.05), with no change in exocytosis rate.

**Conclusion:** In conclusion, these results suggest that therapeutic concentrations of cyclosporin A and tacrolimus, inhibit glucose uptake by removing GLUT4 from the cell surface via increased endocytosis and this is independent of the insulin signalling cascade. It is believed that the described effects of immunosuppressive agents on adipocytes and other insulin-sensitive cells may contribute to the development of insulin resistance and new-onset diabetes associated with immunosuppressive therapy in organ-transplanted patients, particularly in regard to those known as calcineurin inhibitors.

Clinical Trial Registration Number: AD 336-07

Supported by: FCT – The Portuguese Foundation for Science and Tech (SFRH/BD/41044/2007)

## 550

### Flavonoids like apigenin and luteolin induce FOXO1 translocation and differential gene expression patterns in human cells

C. Bumke-Vogt<sup>1,2</sup>, M.A. Osterhoff<sup>2,1</sup>, V. Guzman-Perez<sup>1</sup>, S. Schiess<sup>1</sup>, A. Borchert<sup>1</sup>, V. Baehr<sup>2</sup>, A.F.H. Pfeiffer<sup>2,1</sup>

<sup>1</sup>Clinical Nutrition, German Institute of Human Nutrition, Potsdam-Rehbrücke, Nuthetal, <sup>2</sup>Endocrinology, Diabetes and Nutrition, Charité – University Medicine Berlin, Germany.

**Background and aims:** Modulation of insulin signalling by nutritional components is a feasible method to prevent insulin resistance, increase of gluconeogenesis and the development of type 2 diabetes. We screened micronutrients by analyses of FOXO1 translocation as a mediator of insulin effects. To this end we established a stably transfected human cell line (U-2OS-FOXO1-

GFP) with constitutive expression of FOXO1 with a C-terminal GFP-tag for fluorescent-microscopic detection. Micronutrients with potential FOXO1 translocation activity were further analysed for modulating expression of FOXO1 target genes in human hepatoma cells (HepG2) which express gluconeogenic, antioxidative, and cell cycle modulating genes.

**Materials and methods:** Cell cultures of U-2OS-FOXO1-GFP were treated with natural compounds of plant extracts for 2h in a range of 1 to 100µmol/L. Cells were fixed and stained with DAPI for defining nuclear areas during fluorescence microscopy followed by segmentations of cells for intracellular FOXO1-GFP localization. FOXO1 translocation was determined by calculating the ratio of GFP intensities in nuclear and cytoplasmic areas. Reversibility of FOXO1 nuclear translocation was examined in the presence of insulin 100nmol/L. Additionally intracellular trafficking of FOXO1 was visualized using live cell imaging. Gene expression profiling was performed with HepG2 cells. Following starvation cells were treated with 0.5 to 100µmol/L of apigenin and luteolin for 2h and 24h, respectively. RNA was extracted and gene expression determined by qRT-PCR for phosphoenolpyruvatecarboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), catalase (CAT), superoxide-dismutase (SOD), cyclinG2 (CCNG2), cyclin dependent kinase inhibitors 1A/B (p21CIP/p27KIP), and GADD45 for growth arrest and DNA damage repair.

**Results:** Apigenin induced a significant dose dependent nuclear translocation of FOXO1 within the range of 2 to 100µmol/L while the induction by luteolin was already significant from 1 to 100µmol/L. This nuclear import of FOXO1 was reversed by stimulating cells with insulin 100nmol/L. Despite FOXO1 accumulation in the nuclei by apigenin and luteolin, a dose dependent decrease of mRNA expression after 24h was obtained for PEPCK (being significant for apigenin A ≥ 10µmol/L, luteolin L ≥ 20µmol/L), G6Pase (A ≥ 10µmol/L, L ≥ 20µmol/L), CAT (A ≥ 10µmol/L, L ≥ 20µmol/L), SOD (A ≥ 10µmol/L, L ≥ 20µmol/L), CCNG2 (A ≥ 10µmol/L, L ≥ 30µmol/L), p27KIP (A ≥ 20µmol/L, L ≥ 25µmol/L), whereas p21CIP and GADD45 were induced with apigenin 10µmol/L and luteolin 20µmol/L. After 2h treatment with flavonoids downregulation was less pronounced for PEPCK, CyclinG2 and p27KIP. CAT and SOD were not modulated and an upregulation of p21CIP and GADD45 was not detectable in short term expression.

**Conclusion:** Apigenin and luteolin induce an intranuclear accumulation of the transcription factor FOXO1. The FOXO1 targets p21CIP leading to G1 arrest and GADD45 inducing G2 delay and DNA-repair are upregulated by both flavonoids facilitating antiproliferative effects. In contrast, FOXO1 targets like PEPCK and G6Pase responsible for gluconeogenesis are downregulated by apigenin and luteolin with a potential antidiabetic effect.

Supported by: ProFIT-Programm: Förderung von Forschung, Innovationen und Technologien IBB

## PS 033 In vivo insulin action in animals

551

### Transgenic overexpression of Nrf2 ameliorates oxidative stress and several components of the metabolic syndrome in the spontaneously hypertensive rats

O. Oliyarnyk<sup>1</sup>, H. Malinska<sup>1</sup>, L. Kazdova<sup>1</sup>, M. Pravenec<sup>2</sup>;<sup>1</sup>Department of Metabolism and Diabetes, IKEM, <sup>2</sup>Institute of Physiology, Academy of Sciences, Prague, Czech Republic.

**Background and aims:** Nuclear factor-erythroid 2-related factor-2 (*Nrf2* or *Nfe2l2*) is a key transcription factor responsible for constitutive and inducible expression of ARE (antioxidant response element)-regulated genes of antioxidant enzymes. To test the role of this transcription factor in the pathogenesis of metabolic disturbances associated with metabolic syndrome, we investigated the effect of *Nrf2* transgenic expression in spontaneously hypertensive rats (SHR) on the activity of antioxidant enzymes in liver, myocardium, aorta and renal cortex, lipid peroxidation, and carbohydrate and lipid metabolism. **Materials and methods:** Transgenic line of SHR rats was derived by microinjecting zygotes with the mix of the Sleeping Beauty (SB) construct containing *Nfe2l2* cDNA under control of the CAGGS universal promoter and the SB100X transposase. Activity of antioxidant enzymes was measured using kits of Cayman Chemicals, MI, USA.

**Results:** The *Nrf2* transgene was significantly expressed in multiple tissues which was associated with tissue-specific activation of antioxidant enzymes. Compared to SHR controls superoxide dismutase (SOD) activity was elevated in renal cortex (0.060±0.004 vs. 0.039±0.003 U/mg protein, *p*<0.01). Glutathione peroxidase (GSH-Px) activity was increased in plasma, liver, myocardium and aorta (Table), glutathione transferase (GST) was activated in all investigated tissues. Glutathione (GSH) concentration was increased in plasma (6.55±0.18 vs. 5.53±0.19 μM/ml, *p*<0.01) and renal cortex (15.9±0.9 vs. 11.7±1.4 mM/mg, *p*<0.05). Lipid peroxidation products-thiobarbituric acid reactive substances were significantly reduced compared to SHR controls in plasma (1.070±0.101 vs. 1.701±0.110 nM/mg, *p*<0.001), liver (0.96±0.10 vs. 1.64±0.17 nM/mg, *p*<0.05), aorta (0.471±0.035 vs. 0.632±0.060 nM/mg, *p*<0.05) and renal cortex (0.55±0.04 vs. 0.69±0.05 nmol/mg, *p*<0.05). Transgenic rats exhibited increased basal (19.9±2.3 vs. 36.3±4.4 nmol gl./mg prot./2h, *p*<0.01) and insulin stimulated lipogenesis (90±17 vs. 43±6 nmol gl./mg prot./2h, *p*<0.05) measured as incorporation of <sup>14</sup>C-U- glucose into epididymal fat triglycerides and adrenalin stimulated lipolysis (5.2±0.7 vs. 3.2±0.6 μM/g NEFA, *p*<0.05) which suggests higher metabolic activity of adipose tissue. In addition, insulin stimulated incorporation of glucose into diaphragm glycogen was significantly increased (313±48 vs. 151±35 nM gl./g/2h, *p*<0.05) in transgenic rats.

**Conclusion:** Results indicate that *Nrf2* transgenic expression in SHR rats ameliorate oxidative stress in plasma, liver, myocardium, aorta and kidney and improved some features of the metabolic syndrome. Given the role of *Nrf2* as a regulator of antioxidant system, insulin sensitivity and lipid homeostasis, the pharmacological manipulation of *Nrf2* might be beneficial in treatment metabolic disorders related to insulin resistance.

Antioxidant enzymes activities after transgenic expression of Nrf2.

Tissue	SHR	SHR Nrf2
Plasma GSH-Px, u/	271±19	345±8**
Liver GSH-Px, U/mg protein	206±14	288±18**
Myocardium GSH-Px, U/mg protein	121±14	256±24***
Aorta GSH-Px, U/mg protein	66±6	92±6*
Plasma GST, U/ml	5.77±0.49	7.40±0.25*
Liver GST, U/mg protein	116±11	162±15*
Myocardium GST, U/mg protein	22±3	36±3**
Aorta GST, U/mg protein	1.79±0.18	3.22±0.34*
Renal cortex GST, U/mg protein,	48±4	70±7*

\*\*\**p*<0.001, \*\**p*<0.01, \**p*<0.05

Supported by: GAČR 303/10/0505, MZO 00023001

552

### Insulin-receptor substrate-2 (IRS-2) is necessary to maintain the glucokinase and glucokinase regulatory protein expression in mouse liver

E. Blazquez<sup>1,2</sup>, I. Roncero<sup>1,2</sup>, E. Alvarez<sup>1,2</sup>, C. Acosta<sup>3</sup>, C. Sanz<sup>1,2</sup>, P. Barrio<sup>1,2</sup>, V. Hurtado-Carneiro<sup>1,2</sup>, D. Burks<sup>3</sup>;<sup>1</sup>University Complutense of Madrid, <sup>2</sup>Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas (CIBERDEM), Madrid,<sup>3</sup>Centro de Investigación Príncipe Felipe, Valencia, Spain.

**Background and aims:** Because IRS-2 plays an important role on hepatic nutrient homeostasis, we investigate the expression of GK and GKR in liver, with the use of two experimental models of insulin receptor substrate-2 deficiency in mice. One of these models correspond to IRS-2(-/-) deficient mice and the second ones IRS-2(-/-) deficient mice with IRS-2 specifically reintroduced into pancreatic β-cells, using rat insulin promoter [RIP-Irs-2/IRS-2(-/-)].

**Materials and methods:** Plasma glucose and insulin concentrations were determined using a glucose oxidase method or by immunoassay, respectively. Detection of GK and GKR were done by Western blotting. mRNA levels were determined by quantitative real-time PCR. Glucose-phosphorylating activities were measured using a spectrophotometric assay with two glucose concentrations: 0.3mM for low-Km hexokinase activities, a concentration at which GK is essentially inactive, and 30 mM a concentration at which all phosphotransferase activities were measured.

**Results:** In female mice of both groups of IRS-2 KO animals, plasma glucose and insulin concentrations increase significantly. Liver GK activities were significantly lower (*p*<0.0001) in IRS-2 (-/-) mice. This finding was not found in RIP-Irs-2/IRS-2(-/-) animal because no differences with wild type mice were found. No changes in hypothalamus GK activities of IRS-2 (-/-) animals were found. In addition, liver GK and GKR mRNA levels of IRS-2 (-/-) were significantly lower, while in RIP-Irs2/IRS-2(-/-) animals both GK and GKR increased up to the levels of wild type animals. Also, liver protein content of GK was lower in IRS-2(-/-) mice than in wild type animals, but GK levels does not recover in RIP-Irs2/IRS-2(-/-).

**Conclusion:** These results suggest that IRS-2 is important to maintain the activity of liver GK and then the metabolic function of this organ. Also, these findings suggest that the differences between liver and brain GKs may be explained because the expression of hepatic but not brain GK is controlled by insulin. Thus, when β-cell recovers the presence of IRS-2 in KO mice the reduction of GK activity and protein content were not observed.

Supported by: Ministerio de Ciencia e Innovación and CIBERDEM

553

### Bscl2<sup>-/-</sup> mice are lipodystrophic, hypotriglyceridaemic and displayed diabetic cardiomyopathy

X. Prieur<sup>1</sup>, M. Takahashi<sup>2</sup>, M. Nemani<sup>3</sup>, L. Dollet<sup>1</sup>, B. Pillot<sup>1</sup>, C. Mounier<sup>4</sup>, J. Capeau<sup>3</sup>, M. Lathrop<sup>3</sup>, G. Toumaniantz<sup>1</sup>, T. Le Tourneau<sup>1</sup>, B. Cariou<sup>1</sup>, P. Costet<sup>1</sup>, J. Magré<sup>1</sup>;<sup>1</sup>UMR 1087, Nantes, France, <sup>2</sup>CEA, Evry, France, <sup>3</sup>UMR 938, Paris, France,<sup>4</sup>UQAM, Montreal, Canada.

**Aim:** Berardinelli-Seip congenital lipodystrophy (BSCL) is a rare recessive disorder characterized by a near total absence of adipose tissue and severe insulin resistance. Patients with mutations in *BSCL2*, the gene encoding seipin, used to display also cardiomyopathy. We aimed to understand the physiopathological mechanism of BSCL linked to seipin deficiency.

**Method:** *Bscl2*-deficient mice (*Bscl2*<sup>-/-</sup>) were generated and phenotyped. An adipocyte differentiation model has been developed using mouse embryonic fibroblast.

**Results:** *Bscl2*<sup>-/-</sup> mice showed a massive and early loss of adipose tissue, with plasma leptin and adiponectin levels decreased by ≈ 80%. Using embryonic fibroblasts from these mice, we found that lipotrophy is due to a combination of both impaired adipocyte differentiation and increased lipolysis. The adipogenesis impairment was partially rescued with PPARγ agonist rosiglitazone. In addition, the adipocyte differentiation rate improved when phosphatidic acid (PA) production was inhibited with lisofylline P, which suggests that abnormal PA accumulation might partially explain the adipogenesis default caused by seipin-deficiency. *Bscl2*<sup>-/-</sup> mice displayed glucose intolerance, insulin resistance and hepatic steatosis. Surprisingly, *Bscl2*<sup>-/-</sup> mice exhibited hypotriglyceridemia in the fed state (0.74 mg/ml vs 0.99 mg/ml in wild-type mice, *p*< 0.05) that became much more severe after 12 hours of fasting (0.14 mg/ml vs 0.43mg/ml in

wild-type mice,  $p < 0.001$ ). Using kinetic experiments with radiolabelled VLDL, we showed that hypotriglyceridemia is likely due to an acceleration of hepatic VLDL clearance and fatty acid uptake in *Bscl2*<sup>-/-</sup> mice. Finally, using echocardiography, we have shown that *Bscl2*<sup>-/-</sup> mice exhibited impaired left ventricular diastolic function classic of diabetic cardiomyopathy from the age of 2 months that got worse with age. At 10 months old, *Bscl2*<sup>-/-</sup> mice display an increase of heart weight ( $230 \pm 38$  mg vs  $178 \pm 19$  mg in wild-type mice,  $p < 0.05$ ) associated with a tendency in ventricular cavity dilatation.

**Conclusion:** Collectively our data suggest that seipin plays a key role in adipose tissue function and in adipocyte storage capacity and *Bscl2*<sup>-/-</sup> mice constitute a new monogenic model of diabetic cardiomyopathy.

*Supported by:* ALFEDIAM/SFD

## 554

### Effect of chronic caffeine administration on Glut-4, AMPK expression and plasma catecholamines in age-induced insulin resistance

J.F. Sacramento<sup>1</sup>, M.J. Ribeiro<sup>1</sup>, C. Gonzalez<sup>2</sup>, D.D. Antunes<sup>1</sup>, M.P. Guarino<sup>1</sup>, S.V. Conde<sup>1</sup>;

<sup>1</sup>Farmacologia, CEDOC, Faculdade Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal, <sup>2</sup>Bioquímica y Biología Molecular y Fisiología, Facultad Medicina, Universidad Valladolid, IBGM, CSIC, Spain.

**Background and aims:** Our group has recently shown that chronic caffeine intake prevents the development of insulin resistance in high fat and high sucrose rats through a mechanism related with a decrease in sympathetic nervous system activity. We have also showed that chronic caffeine administration reverses IR in aged rats. Herein we investigated if the mechanism behind caffeine reversion of age-induced insulin resistance involves effects on sympathetic nervous system activity, measured as plasma catecholamines, and/or altered expression of GLUT-4, AMPK expression and AMPK activity in insulin-sensitive tissues.

**Materials and methods:** Six groups of rats were used: 3 months old (3M), 3 months caffeine-treated (3MCaf), 12 months old (12M), 12 months caffeine-treated (12MCaf), 24 months old (24M) and 24 months caffeine-treated (24MCaf). Caffeine was administered in drinking water (1g/l) during 15 days. The insulin tolerance test (ITT) was used to measure insulin sensitivity. Catecholamines were measured in serum by HPLC with electrochemical detection. GLUT-4, AMPK $\alpha$ 1 and AMPK $\alpha$ 1 Thr172 expression was determined by Western Blot in skeletal muscle and adipose tissue and normalized to loading protein.

**Results:** The decrease in insulin sensitivity induced by aging was reversed by chronic caffeine intake. Circulating catecholamines were diminished at 12M and 24M rats by 27.2 and 63.8%, respectively and caffeine intake did not alter those values. Glut4 expression decreased by 60.5% in skeletal muscle in 24M rats and chronic caffeine intake restored Glut4 to control levels. AMPK  $\alpha$ 1 expression in skeletal muscle significantly decreased in both 12M and 24M rats by 59.8% and 58.6% respectively, and chronic caffeine intake did not modify those values. Furthermore, caffeine intake did not change AMPK activity in skeletal muscle measured as AMPK $\alpha$ 1 Thr172 expression.

**Conclusion:** Chronic caffeine intake reverses age-induced insulin resistance in rats, an effect that was independent of sympathetic nervous system activity and AMPK expression and activity in skeletal muscle. Our results suggest that the effect of caffeine on insulin sensitivity in aged rats involves an increase in skeletal muscle glucose uptake.

*Supported by:* FCT-PTDC/SAU-ORG/111417/2009

## 555

### Effect of reducing PP2A expression in central and peripheral insulin signalling

P.K. Picardi, J. Caldeira, R. Bortolatti, A. Caricilli, M. Saad; Morphology and Pathology, Faculty Medicine of Jundiaí, Brazil.

**Background and aims:** Protein phosphatase 2A (PP2A) is a multimeric serine/threonine phosphatase which has multiple functions. However, the potential involvement of PP2A in insulin's metabolic signaling pathway is presently unknown. Brain insulin resistant state is characterized by disturbances in transducing the signal from IR/IRS/AKT, changing feeding behaviour and body weight. We showed that diet induced obese rats (DIO) have a marked increase in PP2A protein expression in the hypothalamus.

**Materials and methods:** Thus, we generated a selective, transient reduction in PP2A by infusion of an antisense oligonucleotide designed to blunt the

expression of PP2A in rat hypothalamic areas surrounding the third ventricle in control and obese rats. Insulin signaling was evaluated by western blot.

**Results:** The selective decrease in hypothalamic PP2A resulted in decreased food intake, reduced body weight, reduced adiposity after high-fat feeding, improved insulin action and signaling in hypothalamus and muscle. Central and peripheral insulin signaling was increased by phosphorylation of AKT in PP2A ASO treated rats. No significant changes in the activation of the IR and IRS-1. To assess the impact of hypothalamic PP2A down-regulation on the peripheral action of insulin, we performed hyperinsulinemic-euglycemic clamp studies. The insulin action on peripheral glucose uptake was increased.

**Conclusion:** We have demonstrated an important role of hypothalamic PP2A in the modulation of energy balance, insulin action, and glucose metabolism. Thus, the reduction in hypothalamic PP2A should be sufficient to promote an appreciable weight reduction and access to the brain may also be necessary to optimally improve insulin sensitivity and glucose homeostasis.

*Supported by:* FAPESP

## 556

### Inhibition of 72k-5ptase improves insulin signal transduction and corrects defective glucose homeostasis in diet-induced obesity

L.A. Velloso, D. Bertelli, E.R. Roman, E.P. De Arujo; University of Campinas, Brazil.

**Background and aims:** The 72 kDa inositol polyphosphate 5-phosphatase E (72k 5ptase) controls signal transduction through the catalytic dephosphorylation of the 5-position of membrane-bound phosphoinositides. The reduction of 72k 5ptase expression in the hypothalamus, results in improved hypothalamic insulin signal transduction and reduction of food intake and body mass.

**Materials and methods:** Here, we evaluated the tissue distribution and the impact of obesity on the expression of 72k 5ptase in peripheral tissues of experimental animals. In addition, insulin signal transduction and action were determined in an animal model of obesity and insulin resistance treated with an antisense oligonucleotide that reduce 72k 5ptase expression.

**Results:** In lean Wistar rats, 72k 5ptase mRNA and protein are found in highest levels in heart, skeletal muscle and white adipose tissue. In three distinct models of obesity, Wistar rats and Swiss mice fed on high fat diet and leptin deficient ob/ob mice, the expression of 72k 5ptase is increased in skeletal muscle and adipose tissue. The treatment of obese Swiss mice with an anti-72k 5ptase antisense oligonucleotide results in significant reduction of 72k 5ptase catalytic activity, which is accompanied by reduced food intake and body mass and improved insulin signal transduction and action as determined by immunoblotting and clamp studies, respectively.

**Conclusion:** The 72k 5ptase is expressed in several tissues of rodents; obesity is accompanied by an increase on its expression in insulin sensitive tissues while the inhibition of 72k 5ptase reduces body mass and improves whole body insulin action.

*Supported by:* FAPESP

## 557

### Cyclosporine A and sirolimus treatments impair glucose and lipid metabolism in vivo

P.C.M. Lopes<sup>1</sup>, J. Sereno<sup>2</sup>, A. Furhmann<sup>1</sup>, J.R. Pedro<sup>1</sup>, F. Reis<sup>2</sup>, E. Carvalho<sup>1,3</sup>; <sup>1</sup>Zoology, Center for Neuroscience and Cell Biology, Coimbra, <sup>2</sup>Medicine Faculty, Institute of Pharmacology & Experimental Therapy, Coimbra, <sup>3</sup>The Portuguese Diabetes Association (APDP), Lisbon, Portugal.

**Background and aims:** Immunosuppressive agents (IA) are important in preventing allograft rejection after organ transplantation. Cyclosporine A (CsA), a calcineurin inhibitor, is one of the most effective and used drugs in this field but promotes serious undesirable side effects, including post-transplant diabetes (PTD). Discovering new and more effective drugs has been a challenge. Sirolimus (SRL) is a new option presenting an identical efficacy with apparent less side effects. Nonetheless, their cellular and molecular mechanisms remain to be fully elucidated. The aim of this study was to assess, in Wistar rats, the effects of CsA and SRL treatments for 3 and 9 weeks, as well as, the putative benefits of replacement of CsA with SRL.

**Material and methods:** Rats were treated with CsA (5 mg/kg/day) or SRL (1 mg/kg/day) alone for either 3 or 9 weeks, in combination (CsA for 3 weeks followed by SRL for 6 weeks) or vehicle alone. At the end of treatments, glucose tolerance tests (GTT) were performed. After sacrifice, insulin-stimulated



glucose uptake was measured in isolated epididymal adipocytes, tissues were harvested for PCR and WB analysis and blood was collected to evaluate biochemical parameters.

**Results:** During a GTT, CsA 5 mg/kg/day caused a significant increase ( $287,8 \pm 31,9$  mg/dl vs. vehicle -  $183 \pm 33,9$  at 15 min;  $p < 0,01$ ) in blood glucose at 3 weeks, worsening the effect for the 9 weeks treatments ( $462,2 \pm 51,2$  mg/dl) compared to vehicle ( $375,8 \pm 22,4$  mg/dl,  $p < 0,01$ ) at 15 min, and at 30 min ( $484,2 \pm 50,9$  vs.  $313,4 \pm 15,3$ ,  $p < 0,001$ ). This effect was not significantly different with either SRL or for the combined treatments compared to control. In addition, insulin-stimulated glucose uptake is significantly impaired at both 3 and 9 weeks exposure to either drug compared to vehicle treated animals (presenting a decrease of 20% for the insulin response). In addition, while there is no difference in IRS1 gene expression in fat at 3 weeks, GLUT4 gene expression was decreased for the SRL treatment ( $25,91 \pm 3,3$  vs. vehicle  $52,24 \pm 4,2$ ;  $p < 0,001$ ). At 9 weeks serum triglycerides are increased with CsA treatment ( $196,04 \pm 18,03$  mg/dl) compared to either SRL ( $130,6 \pm 13,6$  mg/dl) or vehicle ( $107,4 \pm 5,7$ ;  $p < 0,05$ ). However, in liver, we observed an increase in lipids for SRL treated animals ( $5,5 \pm 0,6$  mg/dl) compared to vehicle ( $3,7 \pm 0,7$  mg/dl).

**Conclusion:** These results demonstrate that *in vivo* treatment of rats with either CsA or SRL impairs lipid metabolism, insulin-stimulated glucose uptake in isolated adipocytes and CsA causes glucose intolerance. These effects may be responsible for the development of post-transplant diabetes.

Supported by: FCT SFHR/60405/BD/2009; PTDC/SAU-OSM/104124/2008

## PS 034 In vivo insulin action in humans

558

**The triglyceride content in skeletal muscle is associated with hepatic but not peripheral insulin resistance in elderly twins**

L.G. Grunnet<sup>1</sup>, E. Laurila<sup>2</sup>, O. Hansson<sup>2</sup>, P. Almgren<sup>2</sup>, L. Groop<sup>2</sup>, P. Poulsen<sup>3</sup>, A. Vaag<sup>1</sup>;

<sup>1</sup>Diabetes and Metabolism, Rigshospitalet, Copenhagen N, Denmark,

<sup>2</sup>Department of Clinical Sciences, Diabetes and Endocrinology, Malmö University Hospital, Sweden, <sup>3</sup>Novo Nordisk A/S, Bagsværd, Denmark.

**Background and aims:** Intramyocellular triglycerides (IMTG) have been associated with insulin resistance. We investigated the predictors and impact of IMTG on several metabolic parameters including peripheral and hepatic insulin resistance in elderly twins.

**Materials and methods:** Seventy-four elderly same-sex twins underwent hyperinsulinaemic euglycaemic clamps preceded by an intravenous glucose tolerance test. Aerobic capacity ( $VO_{2max}$ ) and body composition (DEXA scan) was determined in all twins. A biopsy from the vastus lateralis muscle was excised in the fasting state. The muscle triacylglycerol content was analysed by biochemical extraction from these biopsies.

**Results:** The percentage of total body fat was the only independent predictor of IMTG content. After adjustment for trunk fat percentages and sex, IMTG level was significantly associated to fasting plasma levels of glucose and insulin as well as hepatic insulin resistance. However, the association was weakened after adjustment for total fat percentages. A 1 SD (34.5 mmol/kg dry weight) increase in IMTG content was associated with a 24% increase of hepatic insulin resistance. No association between IMTG content and peripheral insulin sensitivity was observed.

**Conclusion:** IMTG content is associated with hepatic but not peripheral insulin resistance in elderly twins. We speculate that IMTG content may reflect the general ectopic accumulation of triglycerides including fat in the liver.

Supported by: Danish Diabetes Foundation, FØSU and the Danish Medical Research Council

559

**Diacylglycerol activation of PKC $\theta$  mediates insulin resistance in hyperlipidaemic, obese and type 2 diabetic states**

J. Szendroedi<sup>1</sup>, T. Yoshimura<sup>2</sup>, E. Phielix<sup>1</sup>, M. Marcucci<sup>2</sup>, D. Zhang<sup>2</sup>,

C. Herder<sup>1</sup>, P. Nowotny<sup>1</sup>, G. Shulman<sup>2</sup>, M. Roden<sup>1</sup>;

<sup>1</sup>German Diabetes Center, Duesseldorf, Germany, <sup>2</sup>Yale School of Medicine, New Haven, USA.

**Background and aims:** Lipotoxicity may lead to systemic subclinical inflammation and/or raise myocellular lipid metabolites [ceramides, diacylglycerols (DAGs)] both of which may also explain insulin resistance associated with obesity and type 2 diabetes (T2D). Here we examine the mechanisms underlying (i) lipid-induced muscle insulin resistance in healthy humans (CON,  $n=12$ ,  $30 \pm 5$  years, BMI:  $24 \pm 2$  kg/m<sup>2</sup>) and (ii) insulin resistance in obese, glucose tolerant humans (OB,  $n=5$ ,  $26 \pm 2$  years,  $45 \pm 3$  kg/m<sup>2</sup>) and patients with T2D (T2D,  $n=4$ ,  $61 \pm 1$  years,  $35 \pm 3$  kg/m<sup>2</sup>).

**Materials and methods:** We measured circulating cytokines (TNF $\alpha$ , IL-6, sICAM, adiponectin, RBP-4), intramuscular DAG and ceramide contents, activities of protein kinase C isoforms (PKC  $\beta$ ,  $\delta$ ,  $\theta$ ) before, at 2.5 h and 4 h lipid or glycerol infusion in CON and at baseline in OB and T2D. We measured insulin sensitivity employing hyperinsulinemic-euglycemic clamps combined with [6,6-<sup>2</sup>H<sub>2</sub>]glucose.

**Results:** Whole-body insulin-stimulated glucose disposal was 57% lower during lipid infusion ( $P < 10^{-6}$ ) than during glycerol infusion. Myocellular total DAG content increased ~2 fold ( $P < 0.005$ ) at 2.5 h and remained ~1.4 fold higher ( $P < 0.05$ ) at 4 h of the lipid infusion. Muscle PKC $\theta$  activity increased by 64% ( $P < 0.005$ ) at 4 h of the lipid infusion. Muscle ceramides and circulating cytokines content did not change. Insulin-stimulated glucose disposal was 78% and 88% lower in OB and T2D (both  $P < 0.005$  vs. CON). Cytosolic DAGs were 180% and 220% greater in OB and T2D than in CON (both  $P < 0.0001$  vs. CON), while muscle ceramide content was not increased compared to CON. Muscle PKC $\theta$  activity was 39% higher in OB and 125% higher in T2D compared to CON ( $P < 0.05$ ).

**Conclusion:** In conclusion, these data support the hypothesis that DAG-induced activation of PKC $\theta$  is responsible for muscle insulin resistance during lipid infusion as well as for the insulin resistance observed in human obesity and type 2 diabetes.

*Clinical Trial Registration Number:* NCT01229059

*Supported by:* DFG SFB 575 TP 16B, EFSD Lilly Grant, Schröder and Schmutzler Stiftung

## 560

### One week of treatment with the IL-1 receptor antagonist anakinra improves insulin sensitivity in patients with type 1 diabetes mellitus: results from a clinical trial

E.J.P. van Asseldonk, P.C. van Poppel, D.B. Ballak, R. Stienstra, M.G. Netea, C.J. Tack;

General Internal Medicine, Radboud University Nijmegen Medical Centre, Netherlands.

**Background and aims:** Although typically associated with type 2 diabetes, insulin resistance may also play an important role in the pathophysiology of type 1 diabetes mellitus. Once diabetes has emerged chronically elevated glucose levels further induce insulin resistance (glucose toxicity). We and others have found strong evidence that obesity induces inflammatory changes involving release of the potent pro-inflammatory cytokine Interleukin-1 $\beta$  (IL-1 $\beta$ ) at the level of the fat tissue, which is partly triggered by elevated glucose levels. We hypothesized that treatment with an IL-1 $\beta$  receptor antagonist should improve obesity- and hyperglycemia-associated insulin resistance. Glucose toxicity affects both beta cell function as well as insulin sensitivity. As patients with longstanding type 1 diabetes have no residual beta cell function, any improvement in insulin sensitivity must be a direct effect and cannot be secondary to reduced glucose toxicity leading to improved beta cell function. In the present study we investigated whether the IL-1 receptor antagonist anakinra was able to improve insulin sensitivity in insulin resistant patients with longstanding, type 1 diabetes.

**Materials and methods:** Subjects with type 1 diabetes were treated with anakinra 100 mg s.c. once daily for 1 week. Before, at the end of the treatment week and four weeks later a set of measurements were performed. Inclusion criteria: age  $\geq 18$ ,  $\leq 65$  yrs, type 1 diabetes  $> 5$  years, BMI  $\geq 25$  kg/m<sup>2</sup> insulin requirement  $> 0.5$  IE/kg bodyweight and HbA1c  $> 7.5\%$  with stable glycemic control. A total of 14 patients were included in the study. Insulin sensitivity (primary endpoint) was evaluated using a euglycemic hyperinsulinemic clamp. Patients recorded at least four times daily glucose levels and insulin usage before, at the end of the treatment period and four weeks later.

**Results:** All subjects (F:M = 4:10) completed the study. The subjects did not experience any side effect. Average BMI at baseline was  $31 \pm 1$  kg/m<sup>2</sup> and this did not change during the study. Insulin sensitivity numerically improved after 1 week of anakinra, which effect was sustained over the 4 weeks after the intervention (glucose disposal rate before anakinra treatment  $34.4 \pm 3.7$ , directly after anakinra  $39.0 \pm 4.0$  (P vs baseline 0.22), four weeks after anakinra  $39.3 \pm 3.2$   $\mu$ mol/kg/min (P vs baseline  $< 0.05$ )). Patients reported significantly lower glucose levels at the end and four weeks after anakinra treatment, while using significantly less insulin (mean glucose level at baseline  $9.73 \pm 0.49$ , directly after anakinra  $8.50 \pm 0.43$  (P vs baseline  $< 0.01$ ), four weeks after anakinra  $8.29 \pm 0.59$  mmol/l (P vs baseline  $< 0.05$ ), insulin usage at baseline  $80 \pm 9$ , at the end of anakinra treatment  $68 \pm 7$  (P vs baseline  $< 0.05$ ), four weeks after anakinra  $68 \pm 8$  IE/day (P vs baseline  $< 0.05$ ). HbA1c was  $8.57\%$  at baseline and did not change during the study. Leukocyte and neutrophil counts significantly decreased at the end and four weeks after anakinra treatment.

**Conclusion:** One week of treatment with the IL-receptor antagonist anakinra improves insulin sensitivity and glycemic control in subjects with type 1 diabetes mellitus, which effect is sustained for at least four weeks. These results support an important role for IL-1 in insulin resistant type 1 diabetes and suggest that interventions aimed at blocking its effects may be beneficial.

*Clinical Trial Registration Number:* NCT01285245

*Supported by:* EFSD Novo Nordisk grant, NWO Vici grand for MGN

## 561

### Impact of low birth weight on insulin signalling in muscle precursor cells

N. Schultz<sup>1</sup>, M. Friedrichsen<sup>1</sup>, C. Broholm<sup>1</sup>, B. Mortensen<sup>2</sup>, B. Pedersen<sup>3</sup>, A. Vaag<sup>1</sup>;

<sup>1</sup>Department of Endocrinology, Rigshospitalet, Copenhagen, <sup>2</sup>Steno Diabetes Center, Gentofte, <sup>3</sup>Centre of Inflammation and Metabolism, Rigshospitalet, Copenhagen, Denmark.

**Background and aims:** Low birth weight (LBW) is a marker of an adverse intrauterine environment and is associated with an increased prevalence of Type 2 Diabetes (T2D). Insulin resistance in skeletal muscle represents a key player in T2D development and has previously been associated with birth weight. Studies of muscle biopsies obtained from young men with LBW documented a range of molecular changes relevant to risk of T2D including reduced expression of genes and proteins involved in insulin signaling, altered fiber type composition and more recently differential epigenetic changes, indicating more profound and fundamental changes of their muscle cell functions. We hypothesize that the metabolic and structural changes observed in skeletal muscle of individuals with LBW is associated with an altered function of muscle precursor cells.

**Materials and methods:** Young males with either LBW ( $\leq 10^{\text{th}}$  percentile, n=10) or normal birth weight (NBW) ( $50^{\text{th}} \leq \text{birth weight} \leq 90^{\text{th}}$  percentile, n=9) were studied. Clinical and metabolic characteristic were obtained, and skeletal muscle biopsies were taken. Primary human differentiated myotubes were established from isolated muscle precursors cells, and their differentiation potential and insulin-signaling were investigated. Gene expression of several molecular markers of muscle precursor cell differentiation, including MyoD, Myogenin and Myosin Heavy Chain, were analyzed by rtPCR. Insulin signaling was evaluated by western blotting of p85 and Akt (total Akt and pAkt<sup>Thr308</sup>).

**Results:** LBW subjects displayed a lower weight, height and lean body mass, compared to NBW subjects. The insulin response in the cultured human myotubes, calculated as the fold change of Akt-Thr308 phosphorylation (pAkt<sup>Thr308</sup>) from basal levels, was reduced in LBW individuals (p=0.01). No significant difference in basal and insulin stimulated p85 and Akt protein levels were seen between the two groups. No difference in the differentiation potential between the LBW and NBW group was seen.

**Conclusion:** The findings support the view of fundamental molecular changes of cell functions including defective insulin signaling at the level of pAkt<sup>Thr308</sup> in LBW subjects, contributing to the decreased insulin sensitivity and increased risk of T2D in LBW individuals.

*Supported by:* H-A-2009-040

## 562

### Iron metabolism-related gene expressions in human adipose tissue in association with obesity and insulin sensitivity

J.M. Fernández-Real<sup>1</sup>, J. Moreno-Navarrete<sup>1</sup>, V. Catalan<sup>2</sup>, F. Ortega<sup>1</sup>, J. Gomez-Ambrosi<sup>2</sup>, M. Moreno<sup>3</sup>, M. Serrano<sup>1</sup>, W. Ricart<sup>1</sup>, C. Diéguez<sup>3</sup>, G. Frühbeck<sup>2</sup>;

<sup>1</sup>Hospital of Girona, <sup>2</sup>Clínica Universitaria de Navarra, Pamplona,

<sup>3</sup>University of Santiago de Compostela, Spain.

**Background and aims:** Recent studies have shown a strong link among iron, obesity-related metabolic disturbances and insulin resistance. To the best of our knowledge, no studies have explored the gene expression of iron related genes in human adipose tissue in association with insulin resistance. To gain insight in the possible effects of iron in adipose tissue physiology, we aimed to investigate iron related genes in human adipose tissue according to obesity, insulin resistance and type 2 diabetes.

**Materials and methods:** Ferroportin 1 (FPN1), transferrin (TF), L-ferritin (FTL) and iron regulatory protein 1 (IRP1) expression (Real Time PCR) were analyzed in three independent cohorts of subjects with different degrees of obesity: cohort 1 [91 visceral (VAT) and 89 subcutaneous (SAT) adipose tissue samples]; cohort 2 [69 VAT samples]; and in a third cohort of morbidly obese subjects with different degrees of insulin action (measured using euglycemic clamp) [32 paired SAT and VAT samples]. Circulating levels of total and unsaturated transferrin were also measured.

**Results:** TF, IRP1, FTL and FPN1 gene expression were detected at high levels in both SAT and VAT. In the first and second cohort, in both VAT and SAT, TF and IRP1 gene expression were significantly and negatively associated with BMI, HOMA<sub>IR</sub> and fasting triglycerides and positively associated with adipogenic genes [PPAR $\gamma$ , GLUT4, IRS1, FASN and ACC1 (r coefficients

between 0.35 and 0.66, *p* values between 0.005 and 0.0001). FTL and FPN1 gene expression were significantly and positively associated with BMI, HO-MA<sub>IR</sub> and negatively associated with adipogenic genes [GLUT4, IRS1, FASN and ACC1 (*r* coefficients between -0.31 and -0.57, *p* values between 0.007 and 0.0001)]. Finally, in the third cohort, TF and FTL1 gene expression in VAT were oppositely linked to insulin action (*r*= 0.45, *p*=0.02 and *r*= -0.53, *p*= 0.002, respectively). Interestingly, circulating unsaturated transferrin (iron free) concentration was negatively associated with fasting triglycerides (*r*= -0.30, *p*= 0.006) and glucose (*r*= -0.29, *p*= 0.007), and positively associated with insulin sensitivity (*r*= 0.46, *p*<0.0001).

**Conclusion:** In summary, adipogenic gene expression was positively linked to TF and IRP1 (directly or indirectly associated with iron uptake) while expression levels of FPN1 (an iron export mediator) and FTL (a marker of intracellular iron accumulation) were negatively associated. These data confirms iron as necessary in adipose tissue physiology, while iron in excess could contribute to obesity-associated adipose tissue dysfunction. The strong association of adipose tissue TF gene expression with insulin sensitivity hints at a potential role in the relationship between iron homeostasis and insulin resistance.

*Supported by: ISCIII and CIBER*

## 563

### Novel index of insulin sensitivity from fasting biomarkers: validation in the ACT NOW study

W. Gall<sup>1</sup>, N. Musi<sup>2</sup>, R. DeFronzo<sup>2</sup>, D. Tripathy<sup>2</sup>;

<sup>1</sup>Metabolon Inc, Durham, <sup>2</sup>University of Texas Health Science Center, San Antonio, USA.

**Background and aims:** Although insulin sensitivity measured with the euglycemic hyperinsulinemic clamp remains the gold standard, several simple indices from plasma glucose and insulin concentrations have been proposed as surrogate measures. Of the known indices, the Matsuda insulin sensitivity index correlates well with insulin sensitivity measured with the insulin clamp and has been widely used. We describe a new index of insulin sensitivity derived from fasting insulin and novel insulin sensitivity metabolites (alpha-hydroxybutyrate (α-HB), linoleoyl-GPC (L-GPC), oleate), developed from the RISC clamp study, and validate the results in the ACT NOW study. **Materials and methods:** 602 IGT subjects (FPG=105, 2-h PG [OGTT]=168 mg%) were randomized to PIO (45 mg/day) or placebo (PLAC) and followed for 2.4 years. The Matsuda index of insulin sensitivity was derived from the plasma glucose and insulin concentrations during the OGTT at baseline and at study end. Using mass spectrometry, we measured top-ranking insulin sensitivity metabolites (plasma α-HB, L-GPC, oleic acid), before and after treatment with pioglitazone or placebo. The new index of insulin sensitivity was calculated from an algorithm as part of a novel diagnostic test for insulin resistance, Quantose™-IR, and was validated against the Matsuda insulin sensitivity index.

**Results:** 50 PLAC-treated subjects developed diabetes versus 15 PIO-treated subjects (hazard ratio=0.28; 95% CI= 0.16–0.49; *p*<0.0001). The improvement in Matsuda insulin sensitivity index in PIO-treated subjects ( $3.9 \pm 0.2$  to  $7.6 \pm 0.3$ , *p*<0.0001) was significantly greater than in PLAC-treated subjects ( $3.9 \pm 0.2$  to  $5.2 \pm 0.3$ ) (*p* <0.0001 PIO vs PLAC). At baseline there was no difference in the estimated glucose disposal rate, termed Quantose-M, between the PLAC and PIO groups. PIO treatment led to improvement in Quantose-M ( $5.58 \pm 0.1$  to  $7.31 \pm 0.2$ , *p*<0.001), while no change was observed in the PLAC-treated group ( $5.69 \pm 0.1$  to  $5.69 \pm 0.2$ , *p*=ns). The Quantose-M correlated strongly with the Matsuda insulin sensitivity index both at baseline (*r*=0.791, *p*<0.005) and at study end (*r*=0.829, *p*<0.005). The improvement in Quantose-M also correlated well with the improvement in Matsuda index (*r*=0.783, *p*<0.005) and the decrease in α-HB (*r*=0.464, *p*<0.005), a top biomarker of insulin resistance and glucose intolerance.

**Conclusion:** The Quantose-M insulin sensitivity index, derived from a single fasting plasma sample, correlates strongly with the Matsuda index of insulin sensitivity and parallels the change in insulin sensitivity with a therapeutic intervention, pioglitazone, in IGT subjects. This index provides an excellent measure of insulin sensitivity and may have utility in clinical practice, as well as for large scale research studies.

*Clinical Trial Registration Number: 00220961*

*Supported by: Takeda Pharmaceuticals*

## 564

### Pioglitazone decreases hepatic insulin resistance index and improves hepatic metabolic biomarkers in IGT subjects: the ACTNOW study

A. Gastaldelli<sup>1,2</sup>, D. Tripathy<sup>2</sup>, W. Gall<sup>3</sup>, R.A. deFronzo<sup>2</sup>, ACT NOW Investigators<sup>2</sup>;

<sup>1</sup>Metabolism Unit, CNR Institute of Clinical Physiology, Pisa, Italy, <sup>2</sup>Division of Diabetes, University of Texas Health Science Center, San Antonio, USA,

<sup>3</sup>Metabolon, Inc. Diagnostic, Durham, USA.

**Background and aims:** The aim of the study was to examine the effect of insulin sensitizer pioglitazone (PIO) on hepatic insulin resistance (Hep\_IR) and a set of novel biomarkers associated with hepatic oxidative pathway.

**Materials and methods:** 602 IGT subjects were randomized to PIO (45 mg/day) or placebo (PLAC) and followed for an average of 2.4 years (ACT NOW Study). OGTT screening was performed at baseline and at the end of study in the 441 subjects that completed the study (213 PIO and 228 PLAC). Indices of insulin resistance, both peripheral (by Matsuda index, MI) and hepatic (Hep\_IR, according to Vangipurapu), were calculated from OGTT data. Top-ranking insulin sensitivity metabolites, including plasma alpha-hydroxybutyrate (α-HB), linoleoyl-GPC (L-GPC), oleoyl-GPC (O-GPC), glycine (GLYC), glutamic acid (GLUT), serine, betaine, decanoylcarnitine, and oleic acid, were measured before and after treatment with PIO/PLAC.

**Results:** Of the 441 subjects that completed the study 50 (21.9%) PLAC-treated subjects developed type 2 diabetes versus 15 (7.0%) PIO-treated subjects (*p*<0.005). Pioglitazone significantly improved MI (from  $3.9 \pm 0.2$  to  $7.5 \pm 0.3$ , *p*<0.0001), reduced ALT (from  $30 \pm 1$  to  $24 \pm 1$  U/l, *p*<0.0001), AST (from  $26 \pm 1$  to  $22 \pm 1$  U/l, *p*<0.0007) and induced a small but significant improvement in Hep-IR (-4%, *p*<0.0001 vs placebo). There was no significant difference between groups in plasma metabolite concentrations at baseline. At study end PIO-treated individuals had significantly lower α-HB, oleic acid, GLUT, α-HB/GLYC and higher GLYC, serine, O-GPC and L-GPC (all *p*<0.005 vs PLAC). In PIO-treated subjects the improvement in Hep-IR was associated with improvement in FPG (*r*=0.26, *p*<0.0001), 2h-PG (*r*=0.28, *p*<0.0001), MI (*r*=-0.71, *p*<0.0001), and among the metabolites with reduction in GLYC (*r*=-0.19, *p*=0.01). In a multivariate model, in PIO, after accounting for age, gender and center, decrease in Hep-IR (partial *r*=0.20, *p*=0.02), GLUT (partial *r*=0.13, *p*=0.008), α-HB/GLYC (partial *r*=0.31, *p*=0.001) and increase in MI (partial *r*=0.23, *p*<0.001) were significantly associated with protection from development of type 2 diabetes.

**Conclusion:** PIO improves hepatic and peripheral insulin resistance index and modulates novel oxidative stress biomarkers related mainly to hepatic metabolism which may in part explain the beneficial effects of PIO on hepatic insulin sensitivity.

*Clinical Trial Registration Number: NCT00220961*

## 565

### Distinct effects of insulin on bone marrow metabolism in humans

R. Kiviranta<sup>1,2</sup>, T.T. Pham<sup>3</sup>, J.C. Hannukainen<sup>3</sup>, A. Karmi<sup>3</sup>, H. Immonen<sup>3</sup>, M. Soinio<sup>2</sup>, P. Salminen<sup>4</sup>, P. Nuutila<sup>2,3</sup>;

<sup>1</sup>Medical Biochemistry and Genetics, University of Turku, <sup>2</sup>Department of Medicine, Turku University Hospital, <sup>3</sup>Turku PET Centre, <sup>4</sup>Department of Surgery, Turku University Hospital, Finland.

**Background and aims:** In adults, majority of bone marrow space of long bones is filled with fat tissue. Significant amounts of adipocytes are also present among bone and hematopoietic cells within trabecular bone areas such as vertebral bodies. Despite the advances made in understanding the metabolic activity of other fat depots, very little is known about bone marrow fat, its functional role or glucose metabolism. To characterize bone marrow metabolic activity we measured regional glucose uptake in femoral and vertebral bone marrow during fasting and insulin stimulation in normal weight healthy subjects.

**Materials and methods:** Nine healthy adults (Age  $47 \pm 6$  yr, 2 males, 7 females, BMI  $23.7 \pm 1.9$  kg/m<sup>2</sup>) volunteered for the study. The subjects were imaged with positron emission tomography (PET) using <sup>18</sup>F- fluorodeoxyglucose (<sup>18</sup>F-FDG) tracer to measure glucose uptake (GU) in skeletal muscle, abdominal subcutaneous fat, abdominal visceral fat and vertebral and femoral bone marrow. PET imaging was performed at fasting state and during hyperinsulinemic euglycemic clamp to measure basal and insulin-stimulated glucose uptake.

**Results:** Fasting GU in femoral bone marrow that consists mainly of fat tissue was significantly higher than in subcutaneous (sc) fat ( $4.93 \pm 1.58$  μmol/l/



min in femur vs  $2.82 \pm 0.38 \mu\text{mol/l/min}$  in sc fat,  $p < 0.05$ ) but did not significantly differ from visceral fat. Skeletal muscle GU was 56% higher than that of femoral bone marrow ( $p < 0.01$ ). Interestingly, glucose uptake in vertebral bone marrow that contains bone and hematopoietic cells as well as some adipocytes, was five-fold higher than in femur ( $24.78 \pm 4.59$  vs.  $4.93 \pm 1.58 \mu\text{mol/l/min}$ ,  $p < 0.001$ ). Insulin stimulation during clamp induced a four-fold increase in femoral bone marrow GU ( $20.43 \pm 6.00 \mu\text{mol/l/min}$ ,  $p < 0.001$  vs. fasting state), which remained higher than that of subcutaneous and visceral fat. Skeletal muscle showed an expected robust nine-fold increased in GU to  $69.73 \pm 34.42 \mu\text{mol/l/min}$  that was significantly higher than femoral bone marrow. Surprisingly, insulin did not stimulate glucose uptake in vertebral bone marrow ( $25.98 \pm 3.46$  clamp vs  $24.78 \pm 4.59 \mu\text{mol/l/min}$  at fasting) demonstrating that these two bone marrow compartments differ in their metabolic activity.

**Conclusions:** This study shows that glucose metabolism differs significantly between vertebral and femoral bone marrow. Vertebral bone marrow consists of bone and hematopoietic cells and some adipocytes and the glucose uptake of these cells appears to be insulin independent. Conversely, insulin stimulates glucose uptake in the mainly fatty femoral bone marrow to similar extent than it does in brown fat. Moreover, glucose uptake in femoral bone marrow both in fasting state and during hyperinsulinemic euglycemic clamp is higher compared to either subcutaneous or visceral fat underlining the difference between these fat depots. Thus, our data supports the hypothesis of bone marrow fat as functionally distinct “yellow fat”.

Clinical Trial Registration Number: NCT00793143

Supported by: EU FP6 (18734, Hepadip). Academy of Finland, Sigrid Juselius Foundation

## PS 035 Non-pancreatic molecules and glucose metabolism

566

### Prevention of obesity and insulin resistance by estrogens: crucial role of ER $\alpha$ activation function 2 (AF-2)

S. Handgraaf<sup>1</sup>, E. Riant<sup>1</sup>, A. Fabre<sup>1</sup>, A. Waget<sup>2</sup>, M.-J. Fouque<sup>1</sup>, A. Krust<sup>3</sup>, P. Chambon<sup>3</sup>, R. Burcelin<sup>2</sup>, J.-F. Arnal<sup>1</sup>, P. Gourdy<sup>1,4</sup>;

<sup>1</sup>Equipe9, Institut of Metabolic and Cardiovascular Diseases, Toulouse,

<sup>2</sup>Equipe2, Institut of Metabolic and Cardiovascular Diseases, Toulouse,

<sup>3</sup>IGBMC, Illkirch, France, <sup>4</sup>Diabetology department, Toulouse, France.

**Background and aims:** Estrogens prevent visceral adiposity, insulin resistance and type 2 diabetes through estrogen receptor alpha (ER $\alpha$ ) activation. This receptor is known to modulate the transcription of many genes through two activation functions (AF-1 and AF-2). We recently demonstrated that, although ER $\alpha$ -AF-1 is absolutely required for the uterine proliferative action of estrogens, it is dispensable for their beneficial effects on atherosclerosis but also on body composition and glucose homeostasis.

**Material and methods:** In order to characterize the involvement of ER $\alpha$ -AF-2 domain in the beneficial effects of estrogens on metabolism, ER $\alpha$ -deficient (ER $\alpha^{-/-}$ ) and AF-2 deficient (AF-2<sup>0</sup>) female mice, have been maintained under high-glucose high-fat-diet until 16 weeks of age, and compared to their respective littermates wild type controls in terms of weight gain, body composition, glucose tolerance and insulin sensitivity ( $n = 8-10$  per genotype). Then, to avoid interference with endogenous steroid levels, female mice were ovariectomized at 4 weeks of age and were implanted with subcutaneous pellets allowing a chronic and stable administration of  $17\beta$ -estradiol (E2,  $80 \mu\text{g/kg/day}$ ) for 12 weeks.

**Results:** As compared to their littermates, ER $\alpha^{-/-}$  and AF-2<sup>0</sup> female mice were both characterized by significantly accelerated weight gain (+111% and +82% in ER $\alpha^{-/-}$  and AF-2<sup>0</sup> mice respectively), massive accumulation of adipose tissue in subcutaneous (+79% and +65% in ER $\alpha^{-/-}$  and AF-2<sup>0</sup> mice) and perigonadic (+104% and +74% in ER $\alpha^{-/-}$  and AF-2<sup>0</sup> mice) sites, glucose intolerance (IPGTT, AUC: +68% and +70% in ER $\alpha^{-/-}$  and AF-2<sup>0</sup> mice) and insulin resistance (ITT, AUC: +40% and +27% for ER $\alpha^{-/-}$  and AF-2<sup>0</sup> mice). Since it was confirmed that ER $\alpha^{-/-}$  mice, and in lesser extent, AF-2<sup>0</sup> mice exhibit a significant increase of endogenous levels of sex steroids, we then studied the effects of chronic E2 administration in ovariectomized female mice under high-fat-diet. As expected, E2 exerted a strong preventive effect against fat mass accumulation in wild-type mice (-77% SC and -81% PG,  $p < 0.001$ ), but also on insulin sensitivity (Hyperinsulinemic clamps, GIR: +50%,  $p < 0.001$ ). This beneficial action on body composition and insulin sensitivity was totally abolished in ER $\alpha^{-/-}$  and AF-2<sup>0</sup> mice.

**Conclusion:** As previously demonstrated for the vasculoprotective action of estrogens, we now provide evidence that ER $\alpha$ -mediated beneficial effects on body composition and glucose tolerance are independent of the AF-1 function, but dependent of the AF-2 function of ER $\alpha$ . These results open the way to a specific modulation of ER $\alpha$  aiming to uncouple the metabolic and vasculoprotective actions from undesired proliferative effects on reproductive tissues.

Supported by: Sandra Handgraaf has been supported by a grant from Sanofi Aventis France

567

### Influence of meal timing on glucose metabolism and hyperandrogenism in lean women with polycystic ovary syndrome

J. Wainstein, M. Boaz, Y. Bar-Dayana, D. Jakubowicz;

Diabetes Unit, E. Wolfson Medical Center Tel Aviv University, Holon, Israel.

**Background and aims:** In obese women with polycystic ovary syndrome (PCOS), weight loss improves insulin resistance and hyperandrogenism, resulting in improvement of clinical symptoms. Weight loss is not required in lean PCOS patients; nevertheless, the influence of meal timing and composition on glucose metabolism and hyperandrogenism may have clinical value. In this study we investigate the effects of two isocaloric diets with different meal timing distribution on insulin resistance and hyperandrogenism in lean PCOS patients.

**Methods:** 60 lean PCOS patients (mean age  $29.4 \pm 6.5$  yrs, mean BMI  $22.7 \pm 0.64 \text{ kg/m}^2$ ) were randomized to one of two isocaloric 1500 kcal/day diets

with different meal timing distribution: Subjects in the High Calorie Breakfast Diet (HCB) consumed a 700 kcal breakfast with the macronutrient distribution: carbohydrate:protein:fat: 50:30:20%. Subjects in the High Calorie Dinner diet (HCD) group consumed this identical meal but at dinner. Insulin and glucose response to OGTT and free testosterone were measured at baseline, and after 90 days of dietary intervention.

**Results:** In the HCB group, area under the curve (AUC) glucose decreased from  $708.8 \pm 145.8$  mg/dl/hr at baseline to  $582.6 \pm 134.3$ ,  $p < 0.0001$  at day 90. Similarly, AUC insulin decreased from  $335.3 \pm 161.0$   $\mu$ U/L/hr to  $102.4 \pm 55.2$ ,  $p < 0.0001$ . Additionally, serum testosterone levels decreased from  $3.8 \pm 1.0$  ng/ml vs.  $1.26 \pm 0.68$ ,  $p < 0.0001$ . These values did not change in the HCD group, and change in BMI was not detected in either group.

**Conclusions:** Isocaloric diets with different meal timing distribution influence glucose metabolism and free plasma testosterone levels in lean women with PCOS. High caloric protein and carbohydrate intake at breakfast with reduced intake at dinner results in improved insulin sensitivity and decreased hyperandrogenism. This meal pattern may facilitate the therapeutic management in women with PCOS

Clinical Trial Registration Number: 0048-12-WOMC

## 568

### Impaired hepatic lipid homeostasis plays a key role in the development of glucose intolerance induced by testosterone deficiency

T. Senmaru, M. Fukui, H. Okada, Y. Mineoka, M. Asano, M. Yamazaki, G. Hasegawa, N. Nakamura;

Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Japan.

**Background and aims:** Low serum testosterone concentrations are associated with metabolic disorders such as type 2 diabetes and metabolic syndrome, dramatically illustrated by androgen deprivation in men with prostate cancer. However, the precise mechanism is unknown. On the other hand, previous studies suggest that hepatic triglyceride (TG) accumulation is directly responsible for the subsequent development of hepatic insulin resistance and increment of glucose production. In this study, we sought to determine whether the mechanism by which testosterone deficiency causes metabolic disorders is through impaired hepatic lipid homeostasis.

**Materials and methods:** Male C57BL/6J mice aged 7 weeks were either castrated (CX) or sham-operated (SHAM), and then were fed a high-fat diet (HFD: 507.6 kcal/100 g, fat kcal 56.7%) or a normal diet (ND: 346.8 kcal/100 g, fat kcal 10%) for 3 weeks starting at 8 weeks of age. Glucose tolerance was assessed by IPGTT at 11 weeks of age. Serum lipids concentrations and hepatic lipids contents were measured by HPLC and enzyme assay, respectively. Hepatic gene expression involved in glucose and lipid metabolism was analyzed by quantitative RT-PCR.

**Results:** In ND-fed mice, castration did not affect all metabolic parameters. As expected, HFD-feeding increased body weight, fasting blood glucose and serum insulin levels in both SHAM and CX mice. Fasting blood glucose levels were higher in CX mice than SHAM mice ( $189.4 \pm 11.4$  vs  $121.0 \pm 9.3$  mg/dL,  $p < 0.05$ ,  $n = 7$ ). In IPGTT, blood glucose levels at 30, 60 and 120 min after glucose load were also significantly higher in CX mice. It was noteworthy that, under HFD-feeding conditions, serum TG and VLDL levels were markedly lower in CX mice than SHAM mice (TG:  $18.9 \pm 2.2$  vs  $84.1 \pm 3.7$  mg/dL,  $p < 0.01$ ,  $n = 5$ ). Inversely, hepatic TG contents were significantly higher in CX mice than SHAM mice ( $169.3 \pm 10.8$  vs  $100.5 \pm 15.1$  mg/g liver weight,  $p < 0.05$ ,  $n = 5$ ). These findings were consistent with the results of histological analysis showing increased lipid droplets in the liver of HFD-fed CX mice. Furthermore, the expression of lipogenic genes, including sterol regulatory element binding protein-1c, fatty acid synthase and peroxisome proliferator-activated receptor- $\gamma$ , was up-regulated in HFD-fed mice. The upregulation of these genes was enhanced in CX mice. Inversely, the expression of microsomal triglyceride transfer protein, which is closely related to hepatic VLDL-TG secretion, was down-regulated in HFD-fed CX mice. Moreover, the expression of insulin receptor substrate-2 was down-regulated in those mice. These results of gene analysis suggest that the inhibition of hepatic TG release along with the increment of TG production causes the hepatic TG accumulation in HFD-fed CX mice, leading to the development of hepatic insulin resistance. Testosterone supplementation using slow-release pellets ameliorated glucose intolerance and hepatic steatosis in HFD-fed CX mice.

**Conclusion:** Hepatic TG accumulation due to impaired hepatic lipid homeostasis and the subsequent hepatic insulin resistance may play a pivotal role in the development of glucose intolerance induced by testosterone deficiency. A high-fat diet could be an initiating factor for those changes.

## 569

### Gender difference in bone - energy homeostasis: adiponectin versus testosterone mediated osteocalcin action in women versus men?

B. Buday<sup>1</sup>, P.F. Pach<sup>2</sup>, B. Literáti-Nagy<sup>1</sup>, M. Vitai<sup>1</sup>, Z. Vecsei<sup>1</sup>, É. Péterfai<sup>1</sup>, L. Korányi<sup>1</sup>;

<sup>1</sup>Metabolism, DRC, Balatonfüred, <sup>2</sup>Statormine Consulting, Balatonfüzfő, Hungary.

**Background and aims:** The osteoblast-specific protein osteocalcin (OCN) has been found to be involved in glucose metabolism by increasing insulin secretion and improving insulin sensitivity by upregulating the insulin sensitizing adiponectin gene in adipocytes. Recently OCN was also found to enhance testosterone production in the Leydig cells of male mice so OCN might be linked to energy metabolism through testosterone in men. Based on these data it is plausible to suppose that there is a gender difference between the metabolic effect of OCN in humans. Our aim was to compare the metabolic links of OCN between the two genders since there are only few data about the specific gender related aspects of the bone - energy homeostasis.

**Materials and methods:** Our study included 136 women (aged  $49 \pm 9$  years) and 155 men (aged  $42 \pm 13$  years). Glucose tolerance was assessed by OGTT, categorized as normal (NGT), or impaired (IFG, IGT or drug naïve 2DM) glucose tolerance. All subject underwent an IVGTT to assess insulin secretion and a hyperinsulinemic clamp to measure insulin sensitivity (IS). For clamp index whole body ( $M_1$ ) and muscle glucose uptake ( $M_2$ ) values were used. Glucose, insulin values, basic lipid parameters and adipokine levels were measured besides OCN and testosterone values. Body composition was measured by DEXA.

**Results:** For glucose tolerance both female and male populations were heterogeneous. OCN levels did not differ significantly between the NGT and GI groups (IFG, IGT or 2DM subjects). Testosterone levels were significantly lower in GI than NGT men ( $15.51 \pm 5.21$  vs.  $13.44 \pm 6.24$  ng/ml,  $p = 0.03$ ), while no difference was observed in women. OCN showed a strong positive correlation with the adiponectin levels only in females ( $r = 0.254$ ,  $p = 0.0029$ ) independent of age, BMI and body fat percent (BFP), and a positive correlation with clamp measured IS, i.e.  $M_1$  ( $r = 0.22$ ,  $p = 0.009$ ) and  $M_2$  ( $r = 0.21$ ,  $p = 0.015$ ) values, and negative one with HBA1c ( $-0.27$ ,  $p = 0.0015$ ) and the OGTT glucose AUC ( $-0.18$ ,  $p = 0.04$ ), after the adjustment with age. No correlation was found between OCN and adiponectin levels in men. On the other hand, OCN values showed a strong positive correlation with the testosterone levels in males ( $r = 0.243$ ,  $p = 0.0023$ ) which was also independent of age, BMI and BFP. OCN showed positive association with the  $M_1$  ( $r = 0.23$ ,  $p = 0.004$ ) and  $M_2$  ( $r = 0.202$ ,  $p = 0.01$ ) values and negative with BFP ( $r = -0.199$ ,  $p = 0.013$ ), BFP ( $r = -0.23$ ,  $p = 0.004$ ), waist to hip ratio ( $r = -0.41$ ,  $p < 0.0001$ ) and leptin levels ( $r = -0.185$ ,  $p = 0.02$ ) in men. When data were adjusted with testosterone most of these relationships became weaker and disappeared with  $M_2$ , BMI and BFP.

**Conclusion:** The expected, 'classic' adiponectin mediated insulin sensitizing effect of OCN was only supported in females in our study. In men a testosterone mediated metabolic effect of OCN is more probable based on our data. The possible existence of OCN - adiponectin vs. OCN - testosterone axis in females vs. males has to be further investigated in gender specific studies in the future.

## 570

### Leptin therapy reverses streptozotocin-induced diabetes by suppressing hepatic glucose production and does not require parasympathetic innervation

H.C. Denroche<sup>1</sup>, E. Tudur<sup>1</sup>, U.H. Neumann<sup>1</sup>, S.D. Covey<sup>2</sup>, T.J. Kieffer<sup>1</sup>;

<sup>1</sup>Cellular and Physiological Sciences, <sup>2</sup>Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada.

**Background and aims:** Leptin therapy is capable of normalizing fasting glucose levels in rodent models of insulin-deficient diabetes, independent of increases in circulating insulin levels; yet the mechanisms of this action remain unclear. Hepatic glucose production is a key diabetogenic pathway that contributes to hyperglycemia in insulin-deficient diabetes, and can be down-regulated in part through parasympathetic innervation. We hypothesized that leptin therapy reverses fasting hyperglycemia in insulin-deficient diabetic rodents by suppressing hepatic glucose production, possibly by enhancing parasympathetic tone to the liver.

**Materials and methods:** Blood glucose during a prolonged fast, and liver metabolic parameters were assessed in streptozotocin (STZ)-treated C57BL/6 male mice infused with leptin (STZ-leptin) or vehicle (STZ-vehicle) continu-

ously for 1 week via subcutaneous osmotic pump implants; non-diabetic controls received buffer instead of STZ injection, and sham surgery instead of pump implantation. Secondly, we used this same mode of leptin therapy in STZ-treated C57Bl/6 mice that received either a subdiaphragmatic vagotomy or sham surgery to determine whether leptin therapy could lower blood glucose in STZ-diabetic mice in the absence of hepatic vagal innervation.

**Results:** Ten µg/day leptin administration partially reduced 4 hour fasted blood glucose levels within 5 days of leptin therapy ( $15.3 \pm 0.8$  mmol/l STZ-leptin vs  $24.4 \pm 1.1$  mmol/l STZ-vehicle,  $P < 0.0001$ ) but did not normalize blood glucose concentrations to non-diabetic levels ( $9.0 \pm 0.5$  mmol/l). Interestingly, STZ-diabetic mice treated with leptin showed a robust and progressive fall in blood glucose levels during a prolonged fast from  $16.2 \pm 2.9$  mmol/l at 4 hours, to  $4.6 \pm 0.8$  mmol/l by 15 hours fasting ( $P = 0.004$ ), a total decrease of  $11.6$  mmol/l. In contrast, during this same time frame non-diabetic and STZ-vehicle controls only showed a decrease of  $2.2 \pm 1.0$  and  $3.8 \pm 2.3$  mmol/l respectively, suggesting that leptin therapy dramatically suppresses hepatic glucose production in this model. Moreover, unlike controls, STZ-leptin treated mice were unable to raise plasma ketones during prolonged fasting. Four-hour fasted STZ-leptin treated mice also displayed a ~55% reduction in hepatic *Glut2* expression compared to STZ-vehicle controls, and a severe depletion of hepatic glycogen content ( $0.058 \pm 0.007$  mg/g tissue) compared to STZ-vehicle ( $4.7 \pm 1.2$  mg/g tissue,  $P = 0.003$ ) and non-diabetic controls ( $4.4 \pm 0.9$  mg/g tissue,  $P = 0.0004$ ). We next examined whether the glucose lowering effect of leptin may be mediated by vagal innervation, by determining whether leptin therapy could lower blood glucose in STZ-diabetic mice with a subdiaphragmatic vagotomy. Interestingly, loss of parasympathetic innervation did not block leptin action in this model, as vagotomized mice had a more robust glucose lowering in response to leptin than sham operated controls ( $4.7 \pm 1.6$  mmol/l vs  $18.1 \pm 1.5$  mmol/l on day 3,  $P = 0.0006$ ).

**Conclusion:** Collectively, our data indicate that leptin therapy may lower blood glucose levels in STZ-diabetic mice by suppressing hepatic glucose production, and that the glucose lowering action of leptin in this model of insulin-deficient diabetes is independent of vagal innervation.

*Supported by: Work funded by CIHR. HCD is supported by NSERC. TJK is supported by MSFHR.*

$9.4$  vs  $58.1 \pm 8.3$  years,  $p = 0.001$ ), with earlier diabetes debut ( $40 \pm 10$  vs  $49 \pm 9$  years,  $p = 0.002$ ), higher  $HbA_{1c}$  ( $8.5$  (1.6) vs  $7.3$  (1.6),  $p = 0.014$ ), lower BMI ( $28.7 \pm 4.0$  vs  $33.2 \pm 4.7$ ,  $p = 0.001$ ) and waist circumference ( $100.0$  (10.6) vs  $115.5$  (17.3) cm,  $p < 0.001$ ), and lower 25(OH)D-levels ( $42$  (10) vs  $36$  (14),  $p = 0.015$ ). Ethnicity (beta =  $-6.5$ ,  $p = 0.008$ ) and waist-to-hip ratio (beta =  $-35.5$ ,  $p = 0.012$ ) explained 17% of the variation in 25(OH)D levels.

**Conclusion:** In a bi-ethnic group of patients with type 2 diabetes and hypovitaminosis D we found no correlation of 25(OH)D to insulin resistance or first phase insulin secretion, measured by euglycemic clamp and IVGTT. Despite selecting subjects with 25(OH)D  $< 50$  nmol/l, there were still marked ethnic differences in 25(OH)D levels and anthropometrical measurements, but these differences did not influence insulin sensitivity or insulin secretion. These results do not disprove a relationship between vitamin D and diabetes, but such a relationship, if it exists, is probably more complex than a direct effect on insulin sensitivity or -secretion. It remains to be seen whether a high-dose vitamin D intervention will improve insulin secretion and/or insulin sensitivity in these patients.

*Clinical Trial Registration Number: NCT00992797*

## 571

### Effect of 25-hydroxyvitamin D levels on insulin sensitivity and insulin secretion in subjects with type 2 diabetes. Baseline results from a vitamin D intervention trial

C. Wium<sup>1,2</sup>, H.L. Gulseth<sup>3</sup>, E.F. Eriksen<sup>3,2</sup>, K.I. Birkeland<sup>3,2</sup>;

<sup>1</sup>Hormone Laboratory, Oslo University Hospital, <sup>2</sup>University of Oslo,

<sup>3</sup>Department of Endocrinology, Oslo University Hospital, Norway.

**Background and aims:** Emerging evidence suggests a possible link between vitamin D deficiency and type 2 diabetes, although positive results from epidemiological studies and trials with surrogate end points like the HOMA indexes are proving difficult to confirm in RCTs using gold standard methods like the euglycemic clamp. The DIVINE study is an ongoing intervention trial with vitamin D in patients with type 2 diabetes and vitamin D deficiency. We here present baseline data on insulin sensitivity and insulin secretion in relation to 25-hydroxyvitamin D (25(OH)D) levels.

**Materials and methods:** 28 men and 14 women of Nordic ethnicity, and 9 men and 10 women of South Asian ethnicity, with confirmed type 2 diabetes and 25(OH)D levels below 50 nmol/l were included. They underwent an IVGTT with glucose bolus of 0.3 g/kg body weight, followed by a euglycemic, hyperinsulinemic clamp with insulin infusion of 80 mIU/m<sup>2</sup>/min and measurement of endogenous glucose production (EGP) by tracer dilution method, using 6,6-deuterated glucose. The Acute Insulin Response to glucose (AIRg) was calculated as the incremental AUC for insulin from time 0–10 minutes. Insulin sensitivity was expressed as Glucose Infusion Rate (GIR) in µmol/m<sup>2</sup>/min and Insulin Sensitivity Index (ISI =  $100 \times \text{GIR}/\text{s-Insulin}$ ). Anthropometrical measurements and  $HbA_{1c}$  (%) were also taken. Student's *t* test or Mann Whitney U-tests, as appropriate, Spearman's correlations and multiple regression analyses were performed. A *p*-value  $< 0.05$  was regarded as significant.

**Results:** Median (IQR) AIRg was 135 (294), and mean  $\pm$  SD GIR and ISI were  $785 \pm 448$  and  $73.5 \pm 54.3$  respectively, for the whole group. Deuterated glucose measurements were not yet available for all patients, but preliminary calculations from a subgroup indicated similar results when EGP was included. There was no significant correlation of 25(OH)D to AIRg ( $r = -0.06$ ,  $p = 0.66$ ), GIR ( $r = 0.09$ ,  $p = 0.50$ ) or ISI ( $r = 0.03$ ,  $p = 0.82$ ). There were no significant differences in GIR, ISI or AIRg between the sexes, nor between the two ethnicities, despite South Asians being significantly younger ( $49.7 \pm$



## PS 036 Incretins

572

### Glucagon, GLP-1 and oxyntomodulin increase resting energy expenditure in man

J.I. Bagger<sup>1,2</sup>, T. Viltsbøll<sup>1</sup>, J.J. Holst<sup>2</sup>, F.K. Knop<sup>1</sup>;

<sup>1</sup>Diabetes Research Division, Gentofte University Hospital, Hellerup,

<sup>2</sup>Biomedical Science, University of Copenhagen, Denmark.

**Background and aims:** The satiety hormone oxyntomodulin (OXM) is a pro-glucagon product with body weight lowering effect. It binds to the glucagon-like peptide-1 (GLP-1) receptor and the glucagon receptor, but the mechanisms behind the body weight reducing effect of OXM remain elusive. We measured the resting energy expenditure (REE) during infusions of saline, GLP-1, glucagon, OXM, and GLP-1+glucagon in healthy subjects.

**Materials and methods:** Indirect calorimetry was used to measure oxygen consumption (VO<sub>2</sub>), carbon dioxide production (VCO<sub>2</sub>), respiratory quotient (RQ) and REE after a 3h-continuous double-blinded infusion of either saline, GLP-1 (1 pmol×kg<sup>-1</sup>×min<sup>-1</sup>), glucagon (0.86pmol×kg<sup>-1</sup>×min<sup>-1</sup>), OXM (3 pmol×kg<sup>-1</sup>×min<sup>-1</sup>) or glucagon+GLP-1 (same doses) in 15 healthy male volunteers (age: 22±2 years (mean±SEM); BMI: 23±0.5 kg/m<sup>2</sup>; HbA<sub>1c</sub>: 5.8±0.1%).

**Results:** Plasma glucose (5.1±0.1 mmol/l) and triglyceride (1.0±0.1 mmol/l) concentrations were similar during the calorimetric measurements. REE was significantly (p<0.05) elevated in response to all peptide infusions compared to saline, with OXM eliciting the most pronounced increase in REE (1,850±124 kcal/day (mean±SD) (saline), 2,004±125 (GLP-1), 1,974±109 (glucagon), 2,057±132 (OXM), 2,014±113 (GLP-1+glucagon)). GLP-1 alone and in combination with glucagon elevated RQ significantly compared to saline whereas OXM tended to decrease RQ (0.85±0.02 (saline), 0.86±0.02, 0.86±0.02 (GLP-1), 0.84±0.02 (OXM), 0.88±0.02 (GLP-1+glucagon)).

**Conclusion:** These data suggest that GLP-1, glucagon and OXM increase REE. Also, our RQ results indicate that OXM increases REE by other mechanisms than GLP-1.

Clinical Trial Registration Number: NCT01232244

573

### Preserved postprandial GLP-1 responses in cholecystectomised subjects: no evidence of a physiological role of gallbladder emptying in postprandial GLP-1 release

D.P. Sonne<sup>1</sup>, K.J. Hare<sup>1</sup>, P.C. Martens<sup>2</sup>, J.J. Holst<sup>3</sup>, T. Viltsbøll<sup>1</sup>, F.K. Knop<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine F, Hellerup, <sup>2</sup>Department of Radiology,

Hellerup, <sup>3</sup>Department of Biomedical Sciences, the Panum Institute, Copenhagen, Denmark.

**Background and aims:** Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, bile acids are increasingly being recognized for their function as metabolic regulators. Preclinical studies suggest that gallbladder emptying - via bile acid-induced activation of the G protein-coupled receptor TGR5 in intestinal L cells - plays a significant role in the secretion of the incretin hormone glucagon-like peptide-1 (GLP-1) and postprandial glucose homeostasis. We hypothesized that human gallbladder emptying potentiates postprandial release of GLP-1 and aimed to evaluate whether cholecystectomized patients exhibit impaired postprandial GLP-1 secretion.

**Materials and methods:** Ten cholecystectomized subjects (age: 49±4 years (mean±SEM); BMI: 25±0.4 kg/m<sup>2</sup>; HbA<sub>1c</sub>: 5.9±0.1%) and 10 healthy age-, gender- and BMI-matched control subjects (age: 48±4 years; BMI: 24±0.5 kg/m<sup>2</sup>; HbA<sub>1c</sub>: 5.7±0.1%) were studied. None had any family history of diabetes and all had normal oral glucose tolerance according to 75 g-oral glucose tolerance test (OGTT). Subjects received a 2,200 kJ-standardized fat-rich liquid meal (with acetaminophen for evaluation of gastric emptying) during which blood samples were drawn and duodenal aspirate (for evaluation of intraduodenal bile acid concentrations) was collected through a duodenal tube placed fluoroscopically.

**Results:** Similar fasting plasma glucose levels were observed in the two groups (5.4±0.1 (mean±SEM) vs. 5.2±0.1 mmol/l, P=0.2) whereas postprandial plasma glucose (PPG) excursions were exaggerated in the cholecystectomized group compared to control subjects (1,431±31 vs. 1,313±36 mmol/l×240 min, P=0.023). Similar fasting plasma GLP-1 concentrations were observed in the two groups, and subjects without a gallbladder exhibited pre-

served postprandial responses of GLP-1 compared to the carefully matched healthy control subjects (3,707±400 vs. 3,165±287 pmol/l×240 min, P=0.29). **Conclusion:** In conclusion, cholecystectomized subjects exhibit preserved postprandial GLP-1 responses suggesting that the physiologically important role of gallbladder emptying for postprandial GLP-1 release indicated by preclinical studies is of less importance in humans. Thus, the physiological relevance of potentiation of GLP-1 release via bile acid-induced activation of TGR5 in small intestinal L cells is still questionable in humans.

Clinical Trial Registration Number: 2010-41-4243

Supported by: Novo Nordisk Foundation

574

### CART is expressed in endocrine cells, including K-cells and L-cells, in the human gastrointestinal tract, and CART is released after a carbohydrate-rich meal in humans

L. Shcherbina<sup>1</sup>, E. Östman<sup>2</sup>, J.F. Rehfeld<sup>3</sup>, N. Wierup<sup>1</sup>;

<sup>1</sup>Lund University Diabetes Centre, Malmö, Sweden, <sup>2</sup>Division of Applied

Nutrition and Food Chemistry, Lund, Sweden, <sup>3</sup>Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark.

**Background and aims:** Cocaine- and amphetamine-regulated transcript (CART) is a regulatory peptide with insulinotropic, glucagonostatic, and anorexigenic properties. We have found CART expression in a hitherto unidentified cell population in human and rodent gastrointestinal (GI) tract. The function and regulation of CART in gut endocrine cells are unknown. The aim of this study was to establish the presence of CART producing endocrine cells along the human GI-tract, as well as to unravel the cellular identity of the CART-expressing cells. A secondary aim was to study regulation of CART expression *in vitro* and CART secretion *in vivo* in humans.

**Materials and methods:** Human specimens covering all parts of the GI-tract (n=10) were examined for CART expression using immunohistochemistry and *in situ* hybridization. Double-immunohistochemistry for all major gastrointestinal hormones was used to identify the CART expressing cells. CART plasma levels were assessed in 14 healthy subjects after a carbohydrate-rich meal. In addition, GLUTag cells were used as an *in vitro* model to study regulation of CART gene expression by QRT-PCR.

**Results:** CART expressing endocrine cells were most prominent in the duodenum and in the antrum part of the stomach, whereas such cells were rare in other parts of the GI-tract. In human duodenum CART was expressed in all the main enteroendocrine cell types studied. Thus, of all CART-expressing cells 75±8% were GIP-producing K-cells, 47±0.4% were serotonin-producing EC-cells, and 36±19% were CCK-producing I-cells. CART was also expressed in minor proportions of GLP-1-producing L-cells, as well as in somatostatin-producing D-cells, neurotensin-producing NT-cells and motilin-producing Mo-cells. In the antrum, the majority of the CART expressing cells was identical to gastrin-producing G-cells. Human plasma CART levels were increased after a carbohydrate-rich meal, with the peak at 60 min. In addition, CART levels correlated positively with glucose and insulin levels 15-30 min after the meal. CART expression was evident in GLUTag cells; therefore this cell line was used to study CART gene regulation. CART mRNA in GLUTag cells was increased by 1mM palmitate, but was not affected by either glucose or oleate. On the other hand, addition of GIP, forskolin and IBMX provoked increased CART mRNA levels.

**Conclusions:** CART is expressed in G-cells in the antrum, as well as in Mo-, NT-, EC-, D-, I-, L-, and K-cells in the duodenum. CART mRNA is increased by palmitate, GIP, and cAMP-elevating agents in GLUTag cells. In humans, CART plasma levels are elevated after a meal, however its origin needs further investigation.

Supported by: Swedish Research Council, Novo Nordisk, ALF, the Pahlsson foundation

575

### Effects of advanced glycation end-products on GLP-1 secreting cells

A. Puddu, R. Sanguineti, G.L. Viviani;

Department of Internal Medicine and Medical Specialties, University of Genova, Italy.

**Background and aims:** Glucagon-like peptide-1 (GLP-1), an intestinal hormone involved in glucose homeostasis, is synthesized by posttranslational processing of proglucagon and is secreted from specialized intestinal neuroendocrine cells, in response to dietary nutrients, and to neural and hormonal

signals, like insulin. Hyperglycemia accelerates formation and accumulation of Advanced Glycation End-Products (AGEs), a heterogeneous group of compounds derived from the non-enzymatic reaction of reducing sugars with proteins, lipids or nucleic acids. AGEs are implicated in diabetic complications, in pancreatic  $\beta$ -cell dysfunction, and in induction of insulin resistance. The aim of this study is to investigate whether AGEs can affect function of GLP-1 secreting cells and their responsiveness to insulin.

**Materials and methods:** Glycated serum (GS), prepared by incubating FBS with 50 mmol/l ribose at 37°C for 7 days, was employed to replace part of FBS in the culture medium. The murine enteroendocrine cell line GLUTag cells (kindly provided by Dr Drucker) were cultured for 5 days in the presence of 2 concentrations of AGEs (GS, and 2GS). At the end of the culture: total RNA was extracted and mRNA expression of proglucagon was evaluated by RT-PCR; GLP-1 release was measured following 2 hours incubation with or without 100 nM insulin; insulin-induced phosphorylation of IRS-1, one of the substrate of insulin receptor, was evaluated by Western blot after stimulation with 10 nM insulin for 5'.

**Results:** Exposure of GLUTag cells to AGEs results in increased mRNA expression of proglucagon (GS:  $104.7 \pm 9.24$  %,  $p > 0.05$  vs CTR; 2GS:  $164.7 \pm 8.74$  %,  $p < 0.05$  vs CTR). This change is accompanied to a significant increment of GLP-1 release by cells cultured in presence of AGEs in comparison to cells cultured in standard medium (CTR:  $835.3 \pm 30.75$  fmol/mg prot; GS:  $1023 \pm 6.25$  fmol/mg prot  $p < 0.05$  vs CTR; 2GS:  $1257 \pm 98.96$  fmol/mg prot,  $p < 0.01$  vs CTR). Insulin-induced GLP-1 secretion results in a 1.4 fold increase of GLP-1 secretion in GLUTag cells cultured in standard condition, whereas insulin was unable to stimulate increment of GLP-1 secretion in cells cultured in presence of AGEs (insulin-induced GLP-1 secretion % vs basal: CTR  $141.3 \pm 4.67$ ; GS  $110.5 \pm 9.89$  %,  $p < 0.05$  vs CTR; 2GS  $105.3 \pm 5.31$  %,  $p < 0.05$  vs CTR). Insulin-stimulated IRS-1 phosphorylation at Tyr895 was abrogated by culture with AGEs (insulin-induced IRS-1 phosphorylation % vs unstimulated: CTR  $249 \pm 39$ ; GS  $107 \pm 13$  %,  $p < 0.05$  vs CTR; 2GS  $130 \pm 2$  %,  $p < 0.05$  vs CTR). These data show that AGEs impair GLUTag cell function by: (1) up-regulating the proglucagon gene expression; (2) affecting secretion of GLP-1; and (3) interfering with insulin signal.

**Conclusion:** These results suggest that exposure of GLUTag cells to AGEs, by increasing GLP-1 secretion, could lead the cells to a condition of exhaustion, contributing to the reduced levels of GLP-1 in subjects with T2DM. Moreover abrogation of insulin-stimulated GLP-1 secretion and IRS-1 phosphorylation suggest that AGEs may induce insulin resistance also in GLP-1 secreting cells. It can be speculated that the altered GLP-1 secretion due to AGEs exposure, coupled to reduced insulin-induced GLP-1 secretion, may contribute to the development and worsening of type 2 diabetes and its complications.

## 576

### Cannabinoid receptor 1 activation inhibits secretion of glucose-dependent insulinotropic polypeptide from intestinal K-cells

C.E. Moss<sup>1</sup>, W.J. Marsh<sup>2</sup>, H.E. Parker<sup>1</sup>, E. Ogunnowo-Bada<sup>2</sup>, C.H. Riches<sup>2</sup>, A.M. Habib<sup>1</sup>, M.L. Evans<sup>1</sup>, F.M. Gribble<sup>1</sup>, F. Reimann<sup>1</sup>

<sup>1</sup>Clinical Biochemistry, <sup>2</sup>Medicine, University of Cambridge, UK.

**Background and aims:** Glucose-dependent insulinotropic polypeptide (GIP) is an enteroendocrine hormone which stimulates insulin secretion, and promotes glucose and triglyceride storage. GIP is secreted from K-cells in the duodenum in response to luminal nutrients in a cyclic AMP-dependent manner. As GIP receptor knock-out or pharmacological inhibition and ablation of K-cells have all been shown to be protective in rodent models of obesity, it has been postulated that GIP may serve to link overnutrition to obesity. The aim of this study was to determine if  $G_{q_i}$ -protein coupled receptors could be recruited to lower cyclic AMP levels in and inhibit GIP secretion from K-cells.

**Materials and methods:** Expression of  $G_{q_i}$ -coupled receptors in K-cells (isolated by fluorescent assisted cell sorting from mice expressing yellow fluorescent protein under the control of the GIP-promoter) was detected by Affymetrix 430 2.0 array hybridisation and confirmed by SYBR green qPCR. Hormone secretion from mixed murine intestinal epithelial cultures and in response to an oral glucose tolerance test in rats was assessed by ELISA or MesoScale.

**Results:** Expression analysis revealed expression of the  $G_{q_i}$ -coupled receptor cannabinoid receptor 1 (CNR1) in duodenal K-cells. Isobutyl methyl xanthine (IBMX, 100  $\mu$ M) stimulated GIP secretion from mixed duodenal cultures  $2.9 \pm 0.3$ -fold ( $n=26$ ;  $p < 0.001$  by one way ANOVA with post hoc Bonferroni test), an effect strongly impaired by addition of the cannabinoid receptor 1 (CNR1) agonist methanandamide (mAEA, 10  $\mu$ M,  $89 \pm 9\%$  inhibition,  $n=27$ ,  $p < 0.001$ ). The reverse agonist AM251 (1  $\mu$ M) had no significant

effect on its own but partially prevented mAEA inhibition ( $48 \pm 10\%$  inhibition,  $n=27$ ,  $p < 0.05$ ). Although CNR1 mRNA expression was also detected in small intestinal L-cells, no effect of either mAEA or AM251 was observed on IBMX-stimulated glucagon-like peptide-1 (GLP-1) secretion assayed in parallel from the same samples. Consistent with the in vitro findings, mAEA (10 mg/kg administered 30 minutes before an oral glucose tolerance test in rats) inhibited GIP (area under curve (AUC)  $2900 \pm 400$  ( $n=8$ ) and  $4300 \pm 200$  ( $n=5$ )  $\text{pmol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ ;  $p < 0.05$  by Student's t-test), but not GLP-1 secretion (AUC:  $0.14 \pm 0.02$  ( $n=8$ ) and  $0.13 \pm 0.02$  ( $n=5$ )  $\text{pg} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ , without significantly delaying gastric emptying, monitored by the plasma appearance of acetaminophen co-applied by gavage.

**Conclusion:** GIP secretion can be inhibited by activation of CNR1, demonstrating a previously unreported cross talk between the intestinal endocannabinoid and enteroendocrine systems. CNR1-dependent inhibition of GIP may act to enhance sensitivity to nutrient stimulation, as endocannabinoid levels fall rapidly after feeding. The observed divergence of mAEA effects on GIP and glucagon-like peptide 1 (GLP-1) secretion might allow selective inhibition of K- over L-cell secretion in the treatment of obesity.

Supported by: WT, BBSRC, EU, MRC

## 577

### Lower GIP and glucagon responses in metabolically healthy but obese (MHO) subjects in comparison with at risk obese individuals

S. Calanna, S. Piro, A. Di Pino, R.M. Zagami, F. Urbano, F. Purrello, A.M. Rabuazzo;

Clinical and Molecular Biomedicine, University of Catania, Italy.

**Background and aims:** Obesity is widely acknowledged as a crucial risk factor for metabolic complications. Among obese subjects, there is a phenotype of metabolically healthy but obese (MHO) individuals that shows a favorable cardio- metabolic risk profile. We aimed to evaluate the potential mechanisms underlying the metabolic profile of this subset, including alpha and beta cell function and entero-insular axis.

**Materials and methods:** We studied 129 obese and 24 non-obese subjects. Obese participants were defined as MHO or at risk obese, according to the HOMA-IR index (MHO: lower tertile of HOMA-IR,  $n=43$ ; at risk: upper tertile of HOMA-IR index,  $n=41$ ). We investigated insulin, glucagon and incretin responses after a 120' oral glucose tolerance test (75g- OGTT).

**Results:** During OGTT, MHO individuals showed in comparison with at risk subjects: lower glucose, insulin and C-peptide fasting plasma levels and responses during the OGTT; higher disposition index; lower fasting ( $p=0.004$ ) and at 30' ( $p=0.01$ ) plasma glucose-dependent insulinotropic polypeptide (GIP) levels; lower AUC (0-30) for GIP ( $p=0.008$ ); higher glucagon-like peptide-1 (GLP-1) plasma levels at 90' ( $p=0.02$ ) and 120' ( $p=0.02$ ); lower glucagon plasma levels at baseline ( $p=0.04$ ) and at 30' ( $p=0.03$ ); appropriate glucagon suppression after the oral glucose load.

**Conclusion:** Metabolically healthy but obese subjects show, as well as normal weight individuals, a lower diabetogenic profile by virtue of higher disposition index and unaffected entero-insular axis. At risk obese individuals present increased GIP levels that might play a role in determining increased glucagon secretion and inappropriate glucagon responses after glucose load, thus contributing to impaired glucose homeostasis.

## 578

### Bioactive incretins levels in humans using novel bioassays reveal kinetics that better correlate with insulin levels than traditional assays

T. Yanagimachi<sup>1</sup>, Y. Fujita<sup>1</sup>, J. Honjo<sup>1</sup>, H. Sakagami<sup>1</sup>, H. Kitsunai<sup>1</sup>, Y. Takiyama<sup>1</sup>, A. Abiko<sup>1</sup>, Y. Makino<sup>1</sup>, T.J. Kieffer<sup>2</sup>, M. Haneda<sup>1</sup>;

<sup>1</sup>Department of Medicine, Asahikawa Medical University, Japan,

<sup>2</sup>Department of Cellular & Physiological Sciences, University of British Columbia, Vancouver, Canada.

**Background and aims:** Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretins secreted from the gastrointestinal tract that promote insulin secretion from pancreatic  $\beta$ -cells in a glucose-dependent manner. Both hormones are rapidly cleaved by dipeptidyl peptidase-4 (DPP-4) after secretion into truncated forms that are no longer insulinotropic. Therefore, it is desirable to specifically assay the full-length insulinotropic forms of incretins. Although active GLP-1 levels have been measured by RIA and ELISA, it is unclear if the assays only detect biologically active forms and the measured values are quite variable by each method.

Moreover, currently there is no reliable method to measure bioactive GIP levels. Therefore, we determined active GIP and GLP-1 levels from human blood samples using novel cell-based, receptor-mediated bioassays.

**Materials and methods:** We used the cell lines stably co-transfected with human forms of GIP or GLP-1 receptors, and a cyclic AMP-inducible luciferase expression construct. We incubated the cells in 96-well plates with samples 5 h and then measured luciferase activity via luminometer. We performed a 75 g OGTT in six healthy volunteers and measured total incretin, insulin, C-peptide and glucagon levels by ELISA and active incretin levels by the bioassays in plasma samples.

**Results:** To assess the specificity of the bioassays, we assessed their responsiveness with several synthetic incretin agonists. In the GIP bioassay, GIP (1–42) and short-form GIP (1–30) increased luciferase activity in a concentration dependent. GIP (1–30) yielded approximately 90% activity of GIP (1–42). In the GLP-1 bioassay, GLP-1 (7–36 amide) and exendin-4 produced almost equivalent luciferase activity. In contrast, the GLP-1 analog liraglutide was not as active in the bioassay, with ~18% of GLP-1 (7–36 amide) activity at 1000 pmol/l. In samples collected from human subjects during an OGTT, insulin levels were greatest at 30 min, and C-peptide levels peaked at 60 min after glucose load. Glucagon levels were suppressed by the oral glucose load. Total GIP levels rapidly increased from 0 min ( $18.4 \pm 8.8$  pmol/l) to 15 min ( $52.4 \pm 9.2$  pmol/l) and gradually increased up to 120 min ( $68.8 \pm 11.4$  pmol/l). In contrast, active GIP levels peaked at 30 min (0 min:  $20.7 \pm 3.6$  pmol/l, 30 min:  $89.4 \pm 11.2$  pmol/l) and dropped at 60 min ( $56.0 \pm 3.8$  pmol/l). Notably, active GIP had a positive correlation with the insulin levels ( $r = 0.31$ ,  $p = 0.03$ ). Total GLP-1 levels increased from 0 min ( $16.5 \pm 1.8$  pmol/l) to 30 min ( $24.2 \pm 1.8$  pmol/l) and remained at similar levels up to 120 min ( $25.4 \pm 2.7$  pmol/l). In contrast, active GLP-1 levels reached a peak at 30 min (0 min:  $8.9 \pm 1.4$  pmol/l, 30 min:  $19.1 \pm 5.8$  pmol/l) and dropped at 60 min ( $8.4 \pm 1.0$  pmol/l).

**Conclusion:** The bioassay measuring method for active forms GIP and GLP-1 reveals that the peaks in circulating hormone levels are reached more rapidly than total GIP and GLP-1 levels, and thereafter active incretin levels decreased promptly. This profile of circulating active GIP and GLP-1 better correlated with insulin levels compared to results obtained with the total assays, and thus we propose that the bioassays more accurately reflect the contribution of the incretins in the entero-insular axis.

Supported by: JSPS

## 579

### Glucagon-like peptide-1 receptor activation with liraglutide enhances hippocampal long-term potentiation mediated through Mash1 in obese diabetic (*ob/ob*) mice

V.A. Gault, D. Porter, C. Holscher, P.R. Flatt;

School of Biomedical Sciences, University of Ulster, Coleraine, UK.

**Background and aims:** Studies have shown that consumption of a high fat diet has adverse effects on learning and memory formation in rodents which has been linked to impaired hippocampal function. Activation of the glucagon-like peptide-1 (GLP-1) receptor signaling machinery has been shown to ameliorate the negative effects of obesity-diabetes on learning and memory however, the mechanisms underlying these beneficial actions remain unclear. Therefore, this study examined effects of daily subcutaneous administration of Liraglutide on hippocampal synaptic plasticity, gene expression and metabolic control in adult obese diabetic (*ob/ob*) mice.

**Materials and methods:** Male obese diabetic (*ob/ob*) mice (14 to 16-weeks old; received twice-daily injections (10:30 h and 16:00 h; sc) of Liraglutide (50 nmol/kg;  $n=8$ ) or saline vehicle (0.9% (w/v) NaCl;  $n=8$ ) over a 21-day period. Body weight, non-fasting plasma glucose and insulin concentrations were monitored at 2 to 3-day intervals. Glucose tolerance (18 mmol/kg; *ip*) and insulin sensitivity tests (50 U/kg; *ip*) were performed at the end of the 21-day study period together with measurement of indirect calorimetry, energy expenditure, locomotor activity and food intake using automated Oxymax CLAMS apparatus. Following measurement of metabolic parameters hippocampal synaptic plasticity was determined by *in vivo* electrophysiological recording of long-term potentiation in region CA1. Additionally, hippocampus was excised ( $n=6$ ) and snap frozen prior to extraction of RNA for gene expression analysis

**Results:** Long-term potentiation (LTP) induced in area CA1 was completely abolished in *ob/ob* mice compared with lean controls ( $F=16.2$ ;  $P<0.001$ ). Deleterious effects on LTP were rescued in *ob/ob* mice treated with Liraglutide ( $F=19.1$ ;  $P<0.001$ ) compared to *ob/ob* saline controls. Indeed, Liraglutide-treated mice exhibited superior LTP profile compared to lean controls ( $F=9.2$ ;  $P<0.01$ ). Expression of hippocampal BDNF and NTRK-2 were not significant-

ly different ( $P>0.05$ ), but synaptophysin and Mash1 were decreased in *ob/ob* mice compared to lean littermates. Moreover, treatment with Liraglutide over 21 days increased the expression of Mash1 in *ob/ob* mice (2.0-fold;  $P<0.01$ ). These changes were associated with significantly reduced plasma glucose (21% reduction;  $P<0.05$ ) and markedly improved plasma insulin concentrations (2.1 to 3.3-fold;  $P<0.05$  to  $P<0.01$ ). Liraglutide also significantly reduced the glycaemic excursion following an intraperitoneal glucose load (AUC values: 22%;  $P<0.05$ ) and markedly enhanced the insulin response to glucose (AUC values: 1.6-fold;  $P<0.05$ ).  $O_2$  consumption,  $CO_2$  production, respiratory exchange ratio and energy expenditure were not altered by Liraglutide therapy. Changes observed were not due to simple dietary restriction alone.

**Conclusion:** These data demonstrate that Liraglutide elicits beneficial effects on metabolic control and synaptic plasticity in mice with severe obesity and insulin resistance mediated in part through increased expression of Mash1 believed to improve hippocampal neurogenesis and cell survival.

Supported by: EFSD/GSK grant

## 580

### Liraglutide increases FGF-21 activity and insulin sensitivity after treated with Acrp30 knockdown

J. Yanjun<sup>1,2</sup>, L. Li<sup>1,2</sup>, G. Yang<sup>3</sup>;

<sup>1</sup>College of Laboratory Medicine, Chongqing Medical University, <sup>2</sup>the Key Laboratory of Laboratory Medical Diagnostics in Ministry of Education, Chongqing Medical University, <sup>3</sup>The Second Affiliated Hospital, Chongqing Medical University, Chongqing, China.

**Background and aims:** Glucagon-like peptide (GLP-1) is a gut hormone secreted in response to ingestion of carbohydrates, lipids, and mixed meals from the L-cells located in the distal jejunum, ileum and colon/rectum. Fibroblast growth factor-21 (FGF-21) is a regulator of insulin action on glucose and lipid homeostasis. In previous studies, we have shown that plasma FGF-21 levels were elevated in patients with T2DM and in diabetic patients with ketosis, and were decreased in response to treatment with rosiglitazone. In this study, we hypothesized that 1) the combination of HFD, hyperlipidemia, and hypoadiponectinemia can cause more severe insulin resistance; 2) that the insulin resistance ameliorating effects of liraglutide were, at least in part, due to increased FGF-21 activity.

**Materials and methods:** ApoE KO and adiponectin (Acrp30) knockdown mice fed a high-fat diet (HFD) were treated with liraglutide (10 mg/kg, twice daily) for 16 weeks. Ad-*shGFP* or Ad-*shAcrp30* was injected by tail vein at the end of the 14th and 15th week of HFD feeding. Liraglutide (1mg/kg) or saline was given intraperitoneally twice daily for 8 weeks. Mice were divided into following groups: regular chow (Control group), high-fat diet (HF, group), high-fat diet and Ad-*shGFP* (Ad-*shGFP* group). Hyperinsulinemic-euglycemic clamp studies were performed. Glucose rates of appearance (G<sub>Ra</sub>) were determined with 3-[<sup>3</sup>H] glucose. Whole body G<sub>Ra</sub> and glucose uptake (G<sub>Rd</sub>) were calculated using the non-steady-state equation. Liver tissue was procured for histological examination, real-time RT-PCR and western blot analysis.

**Results:** The combination of HFD, ApoE deficiency, and hypoadiponectinemia resulted in an additive effect on insulin resistance. FGF-21 mRNA expressions in both liver and adipose tissues were significantly increased while FGF-21 receptor 1 (FGFR-1) and  $\beta$ -Klotho mRNA levels in adipose tissue, as well as FGFR-1-3 and  $\beta$ -Klotho mRNA levels in liver were significantly decreased in this model. Liraglutide treatment markedly improved insulin resistance and increased FGF-21 expression in liver and FGFR-3 in adipose tissue, restored  $\beta$ -Klotho and cFos mRNA expression in adipose tissue as well as FGFR-1-3,  $\beta$ -Klotho levels up to the levels observed in control mice and increased cFos expressions in liver. Liraglutide treatment also further increased FGF-21 proteins in liver and plasma.

**Conclusion:** These data suggested that liraglutide might tissue specifically activate FGF-21 activity in adipose tissue through the  $\beta$ -Klotho-FGFR-3 complex and in liver through  $\beta$ -Klotho-FGFR-1-3 complex. And liraglutide not only restored FGF-21 mRNA and protein expression, but also significantly increased FGF-21R,  $\beta$ -Klotho and further increased cFos, which demonstrated that liraglutide maybe have a direct effects on FGF-21 activity. However, after rosiglitazone treatment, the decreased plasma FGF-21 might be just a response to insulin resistance improving. Our study demonstrated that there have been additive effects of HFD, ApoE deficiency, and hypoadiponectinemia on insulin resistance, that FGF-21 resistance was present in this model and that liraglutide decreased the insulin resistance, at least in part, by increasing FGF-21 action.

Supported by: National Natural Science Foundation of China (30971388, 30771037, 81070640)



## 581

**Once weekly GLP-1 receptor agonist albiglutide vs prandial lispro added to basal glargine in type 2 diabetes: similar glycaemic control with weight loss and less hypoglycaemia**

V.L. Fonseca<sup>1</sup>, B. Ahren<sup>2</sup>, F. Chow<sup>3</sup>, J. Gross<sup>4</sup>, R. Ratner<sup>5</sup>, S. Johnson<sup>6</sup>, M. Stewart<sup>7</sup>, F. Yang<sup>7</sup>, L. Leiter<sup>8</sup>, J. Rosenstock<sup>9</sup>;

<sup>1</sup>Tulane University, New Orleans, USA, <sup>2</sup>Lund University, Lund, Sweden, <sup>3</sup>Prince of Wales Hospital, Hong Kong, China, <sup>4</sup>Hospital de Clinicas de Porto Alegre, Rio Grande do Sul, Brazil, <sup>5</sup>MedStar Health Research Institute, Hyattsville, USA, <sup>6</sup>GlaxoSmithKline, Research Triangle Park, USA, <sup>7</sup>GlaxoSmithKline, King of Prussia, USA, <sup>8</sup>St. Michael's Hospital, Toronto, Canada, <sup>9</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, USA.

**Background and aims:** GLP-1 receptor agonists (RA) and prandial insulin are 2 options for advancing T2D uncontrolled on insulin glargine (GL) + oral agents. Harmony 6 was a randomized, open-label, active-controlled trial with primary endpoint at week (wk) 26 and continued treatment to wk 52 that tested once-weekly (QW) albiglutide (ALBI), a GLP-1 RA, vs thrice-daily prandial insulin lispro (LIS) both combined with titrated GL.

**Materials and methods:** Basal insulin-treated patients (mean A1C 8.45%, weight 92 kg) with A1C 7–10% ± oral agents (no sulfonylurea) entered a run-in standardization GL period followed by randomization to ALBI 30 mg QW (n = 282) or LIS (n = 281) and continued on metformin and/or TZD. GL was titrated to target FPG 4.44–7.22 mmol/L. LIS was adjusted per pre-specified algorithm based on BG monitoring. ALBI could be uptitrated to 50 mg QW based on progressively lower A1C targets (> 8% for ≥ wk 8 and < wk 12; ≥ 7.5% for ≥ wk 12 and ≤ wk 26; and ≥ 7.0% for > wk 26 and ≤ wk 48). Hyperglycemia rescue was permitted in both treatment arms based on progressively lower A1C thresholds.

**Results:** A1C was reduced from baseline by −0.82% and −0.66% with ALBI and LIS, respectively, meeting primary non-inferiority endpoint at wk 26 with further A1C decreases by week 52 among completers. Weight changes were sustained to wk 52: ALBI, −0.96 kg; LIS, +1.66 kg (Table). Mean GL dose increased in both groups. Adverse events of interest through wk 52 with ALBI/LIS were: nausea (13.0%/2.1%); vomiting (7.0%/1.4%), injection site reactions (9.5%/5.3%), and hypoglycemia (32.6%/49.8%).

	Week 26 (LOCF) <sup>a</sup>			P Value	Week 52 (Completers) <sup>b</sup>			P Value
	ALBI (n = 279) <sup>c</sup>	LIS (n = 278) <sup>c</sup>	Treatment Difference (95% CI)		ALBI (n = 121) <sup>c</sup>	LIS (n = 141) <sup>c</sup>	Treatment Difference (95% CI)	
A1C, MA Δ from BL, % ± SE	−0.82 ± 0.06	−0.66 ± 0.06	−0.16 <sup>d</sup> (−0.32, 0.00)	< .0001 met non-inferiority	−1.01 ± 0.07	−0.84 ± 0.06	−0.17 <sup>d</sup> (−0.35, 0.02)	.0861
A1C < 7.0%, n (%)	83 (29.7)	70 (25.2)	1.21 <sup>e</sup> (0.78, 1.88)	.3977	54 (44.6)	42 (29.8)	1.487 <sup>e</sup> (0.80, 2.78)	.2301
FPG, MA Δ from BL, mmol/L ± SE	−0.99 ± 0.164	−0.71 ± 0.164	−0.28 <sup>d</sup> (−0.73, 0.18)	.2366	−1.48 ± 0.202	−0.86 ± 0.188	−0.62 <sup>d</sup> (−1.17, −0.07)	.0281
Weight, MA Δ from BL, kg ± SE	−0.73 ± 0.19	+0.81 ± 0.20	−1.54 <sup>d</sup> (−2.09, −1.00)	< .0001	−0.96 ± 0.36	+1.66 ± 0.34	−2.61 <sup>d</sup> (−3.61, −1.62)	< .0001
GL dose, U (Δ from BL)	52 (+5)	50 (+7)			54 (+7)	52 (+8)		
LIS dose, U (Δ from BL)		30 (+14)				35 (+19)		
Hyperglycemia rescue, n (%)	61 (21.6)	66 (23.5)			123 (43.6)	109 (38.8) <sup>a</sup>		.3260 <sup>f</sup>

A1C, glycosylated hemoglobin; ALBI, albiglutide; BL, baseline; CI, confidence interval; FPG, fasting plasma glucose; LIS, insulin lispro; LOCF, last observation carried forward; MA, model-adjusted; SD, standard deviation.

<sup>a</sup> Intent-to-treat population with last observation carried forward.

<sup>b</sup> Observed cases population with the exception of hyperglycemia rescue.

<sup>c</sup> n listed is that of A1C; n varies slightly for different parameters.

<sup>d</sup> ALBI minus LIS.

<sup>e</sup> Odds ratio for ALBI vs LIS.

<sup>f</sup> Log-rank test for overall hyperglycemia rescue for 1 year.

**Conclusion:** QW ALBI may become a simpler and effective therapeutic option in T2D inadequately controlled on basal insulin to improve glucose control with a lower risk of hypoglycemia and significant and persistent weight loss vs thrice daily LIS.

*Clinical Trial Registration Number:* NCT00976391

*Supported by:* GlaxoSmithKline

## PS 037 Gastric bypass surgery

## 582

**The effect of GLP-1 on food intake is lost in trunkally vagotomised participants**

S. Veedefald<sup>1,2</sup>, A. Plamboeck<sup>1,3</sup>, C.F. Deacon<sup>3</sup>, A. Wettergren<sup>2</sup>, L.B. Svendsen<sup>2</sup>, S. Meisner<sup>4</sup>, C. Hovendal<sup>5</sup>, J.J. Holst<sup>3</sup>, T. Vilsbøll<sup>1</sup>, F.K. Knop<sup>1</sup>;

<sup>1</sup>Diabetes Research Division, Department of Internal Medicine, Gentofte Hospital, University of Copenhagen, Hellerup, <sup>2</sup>Department of Surgical Gastroenterology and Liver Transplantation, Rigshospitalet, University of Copenhagen, <sup>3</sup>The Novo Nordisk Foundation Center for Basic Metabolic Research, Department of Biomedical Sciences, Panum Institute, University of Copenhagen, <sup>4</sup>Department of Surgical Gastroenterology, Bispebjerg Hospital, University of Copenhagen, <sup>5</sup>Department of Surgical Gastroenterology, Odense University Hospital, University of Southern Denmark, Hellerup, Denmark.

**Background and aims:** The rapid degradation of glucagon-like peptide 1 (GLP-1) by dipeptidyl peptidase 4 (DPP-4) suggests that endogenous GLP-1 may act locally possibly via the vagus nerve before being degraded. We aimed to evaluate the effect of exogenous GLP-1 on plasma glucose (PG), gastric emptying (GE), insulin secretion and food intake in vagotomised participants.

**Materials and methods:** Ten trunkally vagotomised participants (treated for duodenal ulcer) with pyloroplasty (70±2 years (mean±SEM); fasting PG (FPG): 5.7±0.1 mmol/l), and 10 controls (67±1 years; FPG: 5.5±0.1 mmol/l) received 300-min infusions of GLP-1 (1.2 pmol×kg<sup>−1</sup>×min<sup>−1</sup>) or saline on separate days. A fixed liquid meal containing paracetamol was ingested at t=30 min, and an *ad libitum* meal was served at t=240 min.

**Results:** In vagotomised participants, GLP-1 lowered PG only at t=60 min (9.5±1.0 vs. 7.4±1.0 mmol/l, *p*<0.05) whereas it reduced postprandial PG levels below baseline in controls (5.4±0.1 vs. 4.2±0.2 mM, *p*<0.0001). GE was accelerated in vagotomised participants compared to controls (paracetamol T<sub>max</sub>: 71±16 vs. 152±7 min, *p*<0.001) on the saline day. GLP-1 decreased GE in both groups (paracetamol T<sub>max</sub>: 87±17 vs. 222±5 min, *p*<0.0001), albeit to a lesser extent in vagotomised participants. Insulin secretion was unaffected by GLP-1 in vagotomised participants whereas it was suppressed in controls (due to inhibition of GE by GLP-1). GLP-1 reduced *ad libitum* food intake (367±37 vs. 318±29 g, *p*<0.05) in controls but had no effect in the vagotomised group (249±31 vs. 246±44 g, *p*=NS).

**Conclusion:** The effect of GLP-1 on plasma glucose, gastric emptying, and food intake is diminished in vagotomised participants. This could indicate that the effects of GLP-1 on gastric emptying and food intake are mediated via vagal innervation.

*Clinical Trial Registration Number:* H-D-2008-050

*Supported by:* The Danish Diabetes Association

## 583

**Effect of Roux en-Y gastric bypass and sleeve gastrectomy on glucose metabolism and gut hormone release**

M. Nannipieri<sup>1</sup>, S. Baldi<sup>1</sup>, B. Astiarraga<sup>1</sup>, A. Mari<sup>2</sup>, M. Anselmino<sup>3</sup>, D. Guarino<sup>1</sup>, R. Berta<sup>3</sup>, E. Barsotti<sup>1</sup>, E. Ferrannini<sup>1</sup>;

<sup>1</sup>Internal Medicine, University of Pisa, <sup>2</sup>Bio-Engineering, CNR of Padua,

<sup>3</sup>Bariatric Surgery, Azienda Ospedaliera Universitaria of Pisa, Italy.

**Background and aims:** In morbidly obese patients with type 2 diabetes (T2DM), Roux-en-Y-Gastric-Sypass (RYGB) and Sleeve Gastrectomy (SLV) restore euglycemia after surgery, but the effects on gut hormone release are not completely understood. To evaluate the effects of RYGB and SLV on post-prandial glucose metabolism and gastro-intestinal hormone secretion after an oral MMT in severe obese T2DM patients.

**Materials and methods:** 21 obese T2DM subjects (12 RYGB and 9 SLV) were studied before, 15-d and 1-year after surgery by comparing the response to a MMT preceded by a week of low-calorie intake. β-cell function was assessed by modeling analysis of the C-peptide response to MMT. Plasma ghrelin, polypeptide YY (PYY), and glucagon concentrations were measured during MMT.

**Results:** 15-d and 1-y post-surgery, BMI decreased in RYGB and SLV (42.4±5.9 vs 40.7±5.8 vs 29.0±3.8; 47.6±6.1 vs 45.7±5.9 vs 36.5±6.3 kg/m<sup>2</sup>, respectively, *p*<0.0001 vs baseline). Mean glucose improved (8.8±2.2 vs 8.1±2.7 vs 5.5±1.2, 8.8±1.7 vs 6.9±1.7 vs 5.8±1.6 mmol/l, *p*=0.0002 vs base-

line, respectively) and mean insulin decreased ( $177 \pm 64$  vs  $141 \pm 76$  vs  $115 \pm 56$ ,  $247 \pm 130$  vs  $184 \pm 79$  vs  $140 \pm 63$  pmol/l,  $p=0.005$  vs baseline) in RYGB and SLV, respectively.  $\beta$ -cell glucose sensitivity improved early after surgery ( $28.8 \pm 22.9$  vs  $47.9 \pm 35.5$  vs  $43.8 \pm 25.6$  and  $33.0 \pm 30.9$  vs  $48.8 \pm 39.5$  vs  $45.2 \pm 23.2$  pmol·min<sup>-1</sup>·m<sup>-2</sup>·mM<sup>-1</sup>,  $p=0.02$  vs baseline, respectively). Mean area under the curve (AUC) of ghrelin decreased in both surgeries ( $6552 \pm 4828$  vs  $6223 \pm 5283$  vs  $4155 \pm 1588$  and  $7425 \pm 1917$  vs  $6409 \pm 367$  vs  $2250 \pm 844$  pg/ml<sup>2</sup>·min,  $p=0.0008$ , respectively) and mean ghrelin AUC was directly correlated with glucose AUC changes ( $r^2=0.065$ ,  $p=0.03$ ). After surgery, mean PYY AUC increased in both groups ( $19802 \pm 5745$  vs  $26377 \pm 8335$  vs  $37627 \pm 21088$  and  $11835 \pm 4457$  vs  $17067 \pm 2295$  vs  $19868 \pm 10692$  ng/ml<sup>2</sup>·min,  $p=0.02$ , respectively). Mean PYY AUC was inversely correlated with glucose AUC ( $r^2=0.12$ ,  $p=0.002$ ). Glucagon AUC increased early after surgery, but came back to baseline 1-y later ( $21215 \pm 13707$  vs  $29005 \pm 15421$  vs  $16756 \pm 5747$  and  $15947 \pm 7976$  vs  $24776 \pm 10806$  vs  $14334 \pm 4203$  pg/ml<sup>2</sup>·min, respectively).

**Conclusion:** These findings indicate that both RYGB and SLV are associated with similar improvement in glucose metabolism,  $\beta$ -cell function and glucoregulatory hormone secretion. Increased PYY and enhanced ghrelin suppression are not explained with reduced food intake after surgery.

Supported by: EFSD Clinical Research Grant

## 584

### Short term effects of laparoscopic adjustable gastric banding (LAGB) and Roux-en-Y gastric bypass (RYGB) vs very low calorie diet (VLCD)

A. Iaconelli<sup>1</sup>, M. Gaggini<sup>2</sup>, A. Gastaldelli<sup>2</sup>, G. Mingrone<sup>1</sup>;

<sup>1</sup>Dpt of Internal Medicine, Università Cattolica S. Cuore, Roma,

<sup>2</sup>Cardiometabolic Risk Lab, Institute of Clinical Physiology-CNR, Pisa, Italy.

**Background and aims:** It has been proposed that bariatric surgical procedures could have early weight loss-independent effects on glucose metabolism in obese patients. However, the results could be biased by the low caloric intake in the first days after surgery since very low calorie diet (VLCD) also improves glucose metabolism. Thus, the aims of this study were to evaluate the short term effects (one week) on insulin sensitivity of two different bariatric surgery procedures, i.e., laparoscopic adjustable gastric banding (LAGB) and roux-en-Y gastric bypass (RYGB), and to compare the changes in glucose metabolism observed after surgery vs. results obtained after one week of supervised VLCD in the same patients studied approximately 3 months before surgery.

**Materials and methods:** We studied 20 obese non diabetic subjects ( $13F/7M$ , BMI= $44.1 \pm 0.7$  kg/m<sup>2</sup>) and evaluated insulin sensitivity by hyperinsulinemic-euglycemic clamp ( $40$  mU/min/m<sup>2</sup>) with tracer infusion ( $6,6$ -<sup>2</sup>H<sub>2</sub>-glucose) and beta cell function by IVGTT at the end of the clamp. Subjects were studied on first hospitalization at baseline and after one week of VLCD ( $600$  kcal/day) and then discharged. Approximately 3 months later subjects were re-hospitalized for surgery, LAGB ( $n=10$ ) or RYGB ( $n=10$ ), and re-studied one week after surgical procedure (caloric intake after surgery was similar to first hospitalization). In all three occasions we measured glucose production (EGP), peripheral insulin sensitivity (as glucose disposal normalized by insulin concentration, M/I), hepatic insulin resistance (Hep-IR=EGPxINS), insulin secretion ( $\Delta AUC_{Cpep}/\Delta AUC_{Glucose}$ , from IVGTT at the end of the clamp),  $\beta$ -cell function (disposition index [DI], determined as  $\Delta AUC_{Cpep}/\Delta AUC_{Glucose} \times M/I$ ).

**Results:** After one week of VLCD subjects lost  $-2.1$  kg but without any significant change in either fasting (from  $182 \pm 11$  to  $176 \pm 9$  mg/min) or clamp EGP (from  $21 \pm 8$  to  $26 \pm 5$  mg/min), Hep-IR (from  $26 \pm 3$  to  $25 \pm 4$  mg/kg/min·nM), peripheral insulin sensitivity (M/I from  $4.5 \pm 0.4$  to  $4.9 \pm 0.5$  mg/kg min nM<sup>-1</sup>), or DI (from  $800 \pm 101$  to  $822 \pm 115$ ). On the other hand, one week after surgery we observed a significant decrease in Hep-IR (RYGB:  $-55\%$ ,  $p<0.05$ ; LAGB:  $-29\%$ ,  $p=ns$  vs baseline) and a significant increase in M/I (RYGB:  $+45\%$   $p<0.05$ ; LAGB:  $+20\%$ ,  $p=ns$ ), with a weight loss of  $-5.5$  kg compared to baseline ( $p=ns$  RYGB vs LAGB). No early change was observed in insulin secretion, but RYGB improved hepatic insulin clearance (from  $420 \pm 23$  to  $566 \pm 21$  ml/min kg,  $p<0.05$ ), thus reducing peripheral insulin levels. Insulin clearance did not change after LAGB (from  $454 \pm 35$  to  $455 \pm 35$  ml/min kg,  $p=ns$ ). For this reason, changes in DI ( $+27\%$  vs  $6\%$ ) did not reach statistical significance vs baseline.

**Conclusion:** Early changes in glucose metabolism were mainly due to bariatric surgery per se since one week VLCD did not improve insulin sensitivity. Early metabolic effects of bariatric surgery rely mainly in early improvement of both hepatic and peripheral insulin sensitivity and were more marked in RYGB than in LAGB.

Clinical Trial Registration Number: NCT01063127

## 585

### The effect of vagal nerve blockade via overdrive stimulation on response to a mixed meal in non-diabetic subjects

M. Sathananthan<sup>1</sup>, S. Ikramuddin<sup>2</sup>, P.D. Giesler<sup>1</sup>, J. Laugen<sup>1</sup>, R.A. Rizza<sup>1</sup>, M. Camilleri<sup>1</sup>, C. Cobelli<sup>3</sup>, A.R. Zinsmeister<sup>1</sup>, A. Vella<sup>1</sup>;

<sup>1</sup>Mayo Clinic, Rochester, USA, <sup>2</sup>University of Minnesota, Minneapolis, USA,

<sup>3</sup>University of Padua, Italy.

**Background and aims:** Bilateral truncal vagotomy is associated with weight loss. It is uncertain if the effects of bariatric surgery on weight are mediated in part by partial or complete disruption of the vagal trunks. Vagal nerve stimulators produce reversible Vagal Nerve Blockade (VNB) and have been used as potential therapy for obesity. This provides the opportunity to directly study the effect of the vagus on glucose metabolism and enteroendocrine secretion.

**Materials and methods:** Nine subjects (BMI of  $37.6 \pm 1.2$  Kg/M<sup>2</sup>, fasting glucose of  $5.7 \pm 0.5$  mmol/l) were studied on 3 occasions - each after a 2 week period during which VNB was activated or inactivated in random order. At the end of each period subjects were studied using a standardized triple-tracer mixed meal consisting of 50g of bacon, 2 scrambled eggs and 75g of Jell-O labeled with [<sup>1-13</sup>C]-glucose. [<sup>6-3</sup>H] glucose was infused intravenously to measure the systemic rate of meal appearance (Meal Ra). Infused [<sup>6-3</sup>H<sub>2</sub>] glucose enabled measurement of endogenous glucose production (EGP) and glucose disappearance (Rd). The meal was also labeled with [<sup>111</sup>In-DTPA] to enable measurement of gastrointestinal transit. Insulin action (Si) and  $\beta$ -cell responsiveness indices ( $\phi$ ) were estimated using the oral minimal model.

**Results:** The analysis focused on differences between VNB activation and inactivation. Fasting Ghrelin concentrations were slightly but not significantly lower when VNB was activated ( $590 \pm 64$  vs.  $623 \pm 71$  pmol/l, ON vs. OFF respectively,  $p=0.09$ ). No differences in Ghrelin concentrations were apparent after meal ingestion. Integrated glucose ( $184 \pm 66$  vs.  $232 \pm 75$  mol,  $p=0.24$ ), insulin ( $27.5 \pm 5.2$  vs.  $28.9 \pm 3.0$  nmol,  $p=0.52$ ) and glucagon ( $24.1 \pm 3.3$  vs.  $22.7 \pm 2.3$   $\mu$ mol,  $p=0.27$ ) concentrations did not differ between study days. Fasting EGP, suppression of postprandial EGP and integrated Rd also did not differ (table). Interestingly, peak Meal Ra was higher ( $59.8 \pm 2.6$  vs.  $67.3 \pm 2.1$   $\mu$ mol/kg/min,  $p=0.03$ ) when VNB was turned off. No difference in the time taken to empty 10% ( $GE_{10}$ ) and 50% ( $GE_{50}$ ) of stomach contents. Gastrointestinal transit at 24 hours ( $GC_{24}$ ) was also unchanged (table).

**Conclusion:** We conclude that VNB by overdrive stimulation at the level of the cardia alters peak Meal Ra (perhaps by altering fasting gastric volume) but has no direct effects on subsequent gastrointestinal transit, Ghrelin concentrations, glucose metabolism or insulin secretion and action.

	GE <sub>10</sub> (min)	GE <sub>50</sub> (min)	GC <sub>24</sub>	Fasting EGP ( $\mu$ mol/kg/ min)	EGP Suppression (%)	Integrated Rd (Mol per 6hr)
activated (ON)	80 $\pm$ 10	160 $\pm$ 23	2.2 $\pm$ 0.4	16.2 $\pm$ 1.5	81.2 $\pm$ 1.5	13.2 $\pm$ 0.4
inactivated (OFF)	78 $\pm$ 12	160 $\pm$ 15	2.4 $\pm$ 0.4	16.0 $\pm$ 1.8	85.7 $\pm$ 1.1	13.3 $\pm$ 0.4
p-value	0.70	0.93	0.45	0.86	0.50	0.74

Clinical Trial Registration Number: NCT01173111

Supported by: NIDDK

## 586

### Glucose homeostasis and incretin hormones one year after bariatric surgery in patients with type 2 diabetes mellitus and obesity

G. Nasso<sup>1</sup>, P.P. Cutolo<sup>2</sup>, M. Cotugno<sup>1</sup>, G. Saldalamacchia<sup>1</sup>, R. Lupoli<sup>1</sup>, E. Griffo<sup>1</sup>, G. Vitagliano<sup>1</sup>, G. Riccardi<sup>1</sup>, L. Angrisan<sup>2</sup>, B. Capaldo<sup>1</sup>;

<sup>1</sup>Department of Clinical and Experimental Medicine, University Federico II,

<sup>2</sup>General and Laparoscopic Surgery Unit, San Giovanni Bosco Hospital, Naples, Italy.

**Background and aims:** Bariatric surgery (BS) improves glucose homeostasis in patients with T2DM and obesity. Aim. To evaluate insulin sensitivity, insulin secretion and incretin hormones in patients with T2DM and severe obesity who had undergone laparoscopic gastric bypass (GB) or sleeve gastrectomy (SG).

**Materials and methods:** In 32 T2DM obese patients [14 operated of GB (6 M;  $48 \pm 9$  years  $43 \pm 6$  Kg/m<sup>2</sup>, M $\pm$ SD) and 18 of SG (8 M;  $44 \pm 10$  years;  $47 \pm 8$  Kg/m<sup>2</sup>)] we evaluated insulin sensitivity and insulin secretion by OGTT and incretin response to a mixed meal before and 1 year after BS.

**Results:** At 1 year, weight loss was similar (~ 30%) and 70% of patients in both groups experienced T2DM remission (fasting plasma glucose < 100 mg/dl and HbA<sub>1c</sub> < 6.0 % in the absence of hypoglycemic therapy). Insulin sensitivity (OGIS index) increased by 53% (from 306 ± 92 to 653 ± 221 ml min<sup>-1</sup>m<sup>-2</sup>, p=0.04) after GB and by 57% (from 346 ± 99 to 539 ± 543 ± 112 ml min<sup>-1</sup>m<sup>-2</sup>, p=0.05) after SG. Insulin secretion (Insulinogenic index) significantly increased in both groups (from 0.58 ± 0.09 to 1.2 ± 0.54 ng/mmol, p=0.05 in GB; from 0.47 ± 0.06 to 1.5 ± 0.6, p=0.04 in SG). GLP-1 peak after mixed meal, rather flat in all subjects preoperatively, doubled 1 year after GB (GLP1<sub>130-Ln</sub> from 1.7±0.9 to 3.6±0.7 pM/L BG, p=0.0001) while it remained substantially unchanged after SG (from 1.2±0.5 to 1.8±0.6 pM/L, p=0.13); in contrast, GIP did not change either after GB or after SG.

**Conclusion:** Insulin secretion and insulin sensitivity improved to the same extent with the two interventions, while GLP-1 profile improved only after GB. These data indirectly support the predominance of mechanisms other than incretin release in the remission of T2DM after GB or SG.

## 587

### Fibroblast growth factor 19 (FGF19) is regulated by gastric bypass and mimics the metabolic benefits after the surgery

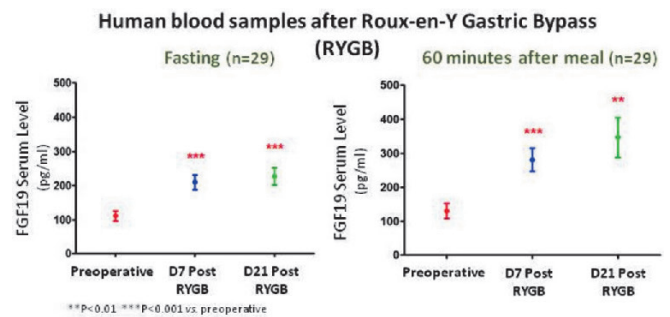
A.M. DePaoli<sup>1</sup>, L. Ling<sup>1</sup>, D.D. Kaplan<sup>1</sup>, M. Saberi<sup>1</sup>, P. Zhang<sup>1</sup>, D. Lindhout<sup>1</sup>, S. Gao<sup>1</sup>, H. Yang<sup>1</sup>, M. Zhou<sup>1</sup>, L.E. Davidson<sup>2</sup>, S.C. Hunt<sup>2</sup>, T.D. Adams<sup>2</sup>, H. Tian<sup>1</sup>; <sup>1</sup>NGM Biopharmaceuticals, South San Francisco, <sup>2</sup>University of Utah, Salt Lake City, USA.

**Background and aims:** The gastric bypass (GB) procedure leads to acute remission of type 2 diabetes (T2D) in many patients before significant weight loss. This metabolic improvement is heralded by both an increase in insulin secretion, perhaps explained in part by GLP-1, and an improvement in insulin sensitivity, at this point unexplained. Our goal has been to discover genes that explain the observations of rapid improvement in glucose homeostasis and long term alterations in body weight that could provide a potential approach to mimicking the beneficial effects of the GB procedure.

**Materials and methods:** (1) Microarray and quantitative RT-PCR analyses were performed using RNA extracted from segments of intestines from Zucker Fatty rats following duodenal-jejunal bypass (DJB) or sham surgery. (2) Serum FGF19 levels were determined in 31 T2D patients preoperatively, and 7 days and 21 days following Roux-en-Y gastric bypass (RYGB) or gastric banding bariatric surgery. (3) Glucose, body weight, serum insulin levels and insulin sensitivity were also determined after FGF19 delivery in *db/db*, diet-induced obese (DIO), *ob/ob* and TALLYHO mice, as well as spontaneously obese and insulin resistant cynomolgus monkeys.

**Results:** (1) FGF15 (murine ortholog of FGF19) was identified as significantly regulated at the mRNA level through a systematic gene expression study of Zucker Fatty rats following DJB, a weight-neutral GB procedure. After surgery, FGF15 mRNA is up-regulated by >150% in the ileum as compared to sham-operated rats. (2) FGF19 fasting and fed levels were significantly increased in obese patients with diabetes at 7 and 21 days post GB compared to their preoperative baseline before surgery. (Figure). (3) FGF19 administration in rodent models of diabetes and obesity led to significantly reduced or normalized glucose in DIO, *ob/ob*, *db/db* and TALLYHO mice. Glucose tolerance was assessed in DIO and *ob/ob* mice with an oral glucose load and was significantly improved (similar to GLP-1). A reduced body weight was seen in DIO and *ob/ob* mice, but not in *db/db* and TALLYHO mice. FGF19 demonstrated a significant improvement in hepatic insulin sensitivity in a euglycemic hyperinsulinemic clamp study in *db/db* mice. (4) Daily subcutaneous administration of FGF19 to spontaneously obese, insulin resistant cynomolgus monkeys led to a rapid improvement in insulin sensitivity independent of weight loss.

**Conclusion:** FGF19 is a secreted GI hormone that is regulated by gastric bypass and may mediate some of the metabolic improvements and sustained body weight loss seen with the GB procedure. Further study of this pathway as a potential therapeutic approach to T2D seems warranted.



## 588

### Hepatic and peripheral insulin sensitivity 1 week and 3 months after Roux-en-Y gastric bypass surgery in subjects with type 2 diabetes and normal glucose tolerance

K.N. Bojsen-Møller<sup>1</sup>, C. Dirksen<sup>1</sup>, S.H. Jacobsen<sup>1</sup>, N.B. Jørgensen<sup>1</sup>, A.K. Serup<sup>2</sup>, D.L. Hansen<sup>1</sup>, D. Worm<sup>1</sup>, J.J. Holst<sup>3</sup>, E.A. Richter<sup>2</sup>, S. Madsbad<sup>1</sup>; <sup>1</sup>Department of Endocrinology, Hvidovre Hospital, <sup>2</sup>Department of Exercise and Sport Sciences, University of Copenhagen, <sup>3</sup>Department of Biomedical Sciences, University of Copenhagen, Denmark.

**Background and aims:** Roux-en-Y gastric bypass (RYGB) improves glucose metabolism in type 2 diabetes (T2D) within days after surgery but whether early changes in insulin sensitivity are part of the physiological mechanism is not known.

**Materials and methods:** We studied 9 obese subjects with T2D (BMI: 38.1±1.8 kg/m<sup>2</sup> (mean ± sem), HbA<sub>1c</sub>: 7.1±0.4%) and 10 obese subjects with normal glucose tolerance (NGT) (BMI: 40.2±0.8 kg/m<sup>2</sup>, HbA<sub>1c</sub>: 5.4±0.1%) before, 1 week (wk) after and 3 months (mo) after RYGB using 4 hours hyperinsulinemic (40 mU/m<sup>2</sup>/min) euglycemic (5.5 mmol/l) clamp combined with infusion of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose to assess hepatic glucose production (HGP). Fat free mass (ffm) was assessed by whole body DEXA scan.

**Results:** Fasting glucose levels decreased in both groups at 1 wk and remained lower after 3 mo (T2D: 8.5±0.8, 6.8±0.4 p=0.05, 5.9±0.3 mmol/l p=0.05; NGT: 5.1±0.1, 4.8±0.2 p=0.02, 4.6±0.1 mmol/l p<0.01). HOMA-IR was significantly reduced in both groups at 1 wk and 3 mo (T2D: 5.2±0.9, 3.9±1.0 p=0.04, 1.7±0.3 p=0.05; NGT: 2.8±0.4, 1.7±0.2 p=0.01, 0.9±0.1 p<0.01). Basal HGP decreased 1 wk after RYGB in T2D (3.7±0.4, 3.0±0.2 mg/kg<sub>ffm</sub>/min p=0.05) whereas no significant change was observed in NGT (3.2±0.3, 2.8±0.1 mg/kg<sub>ffm</sub>/min p=0.25). Peripheral insulin sensitivity measured by M<sub>ffm</sub>/I was unchanged 1 wk after RYGB in both groups but improved at 3 mo (T2D: 11.1±2.2, 14.7±2.8 p=0.09, 24.4±3.6 g/kg<sub>ffm</sub>/min/pmol/l p=0.03; NGT: 18.7±2.8, 16.2±1.6 p=0.16, 27.9±3.0 g/kg<sub>ffm</sub>/min/pmol/l p=0.02) at which time weight loss was 14.0±0.9% (p<0.01) in T2D and 17.5±1.9% (p<0.01) in NGT.

**Conclusion:** Decreased hepatic glucose production was observed 1 wk after RYGB in T2D explaining the early reduction in fasting glucose. Peripheral insulin sensitivity did not improve prior to weight loss but was evident at 3 mo postoperatively. RYGB may have differential effects on tissue insulin sensitivity with early improvements in hepatic insulin sensitivity and later weight-loss dependent improvements in peripheral insulin sensitivity.

Clinical Trial Registration Number: NCT01202526

Supported by: Research program of the UNIK: Food, Fitness & Pharma for Health and Disease



## PS 038 Mitochondria

### 589

#### Elevated mitochondria biogenesis in skeletal muscle is associated with testosterone-induced weight loss

K. Kajita<sup>1</sup>, T. Usui<sup>1</sup>, K. Fujioka<sup>1</sup>, I. Mori<sup>1</sup>, T. Hanamoto<sup>1</sup>, T. Ikeda<sup>1</sup>, H. Okada<sup>1</sup>, K. Taguchi<sup>1</sup>, Y. Uno<sup>1</sup>, H. Morita<sup>1</sup>, M. Shimpuku<sup>2</sup>, T. Sasaki<sup>2</sup>, T. Kitamura<sup>3</sup>, I. Kojima<sup>2</sup>, T. Ishizuka<sup>1</sup>;

<sup>1</sup>General Internal Medicine, Gifu University Graduate School of Medicine,

<sup>2</sup>Institute for Molecular and Cellular Regulation, Gunma University,

Maebashi, <sup>3</sup>General Internal Medicine, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan.

**Background and aims:** Numerous clinical observations have revealed that low serum testosterone level is associated with male obese or type 2 diabetic patients. These results suggest that testosterone prevents obesity in men. Recently, we found that feeding with food containing 0.4% DHEA or testosterone (T) equally decreases body weight and fat weight without affecting food consumption in rats. Moreover, DHEA or T treatment similarly decreases PPAR $\gamma$ , lipoprotein lipase and adiponectin mRNA levels in 3T3-L1 adipocytes, however these treatment did not influence lipolysis and expression levels of SREBP-1 and fatty acid synthase. These results suggest that reduced adiposity by DHEA or T cannot be explained by increased lipolysis and suppressed triglyceride production in adipocyte. Therefore, possible mechanism is increased energy expenditure. In this study, we examined the effect of T on mitochondrial heat production, since little is known about the effect of androgen on mitochondria biogenesis, especially in skeletal muscle.

**Materials and methods:** C57/black mice were housed with or without 0.4% T containing food for 4w, and then O<sub>2</sub> consumption and CO<sub>2</sub> production were measured by indirect calorimetry. Expression of PPAR $\gamma$  coactivator 1 $\alpha$  (PGC1 $\alpha$ ) and mitochondrial protein ATP5B, Cox 4 and cytochrome c levels in various organ were measured with western blot and real time RT-PCR. In addition, effect of T on amount of mitochondria in C2C12 myotube was accessed with Rhodamine 123.

**Results:** Treatment with T significantly decreased body weight and fat weight in C57/black mice, whereas food consumption was not changed. O<sub>2</sub> consumption during 24 hr and light(resting) phase was significantly elevated in the treated mice, while locomotor activity was significantly lower in the treated group. Respiratory quotients, the ratio: eliminated CO<sub>2</sub>/consumed O<sub>2</sub>, were not changed in the treated mice compared with control. These results indicated that testosterone-induced weight loss due to the increased energy expenditure. Furthermore, the fact that O<sub>2</sub> consumption increased despite of decreased locomotor activity indicates that testosterone administration raises basal metabolic rate. Treatment with T increased protein level of PGC1 $\alpha$  in brown adipose tissue (BAT) and skeletal muscle, but not in white adipose tissue and liver. The expression levels of ATP5B and Cox 4 were increased in skeletal muscle by the treatment. Although T increased the expression levels of ATP5B and Cox 4 in BAT, the change was not significant. Treatment with 10 nM T increased the expression level of PGC1 $\alpha$  mRNA, Cox 4 protein level and staining levels with Rhodamine 123 in a concentration depending manner in C2C12 myotube. Since PGC1 $\alpha$  is a key regulator of mitochondrial biogenesis, T-induced upregulation of PGC1 $\alpha$  may lead to increase mitochondrial specific protein levels especially in skeletal muscle.

**Conclusion:** T-induced weight loss is associated with increased basal metabolic rate, which may be explained by the increased PGC1 $\alpha$  expression and mitochondrial biogenesis in skeletal muscle.

### 590

#### Impaired insulin-/IGF1-signalling extends life span by promoting mitochondrial L-Proline catabolism to induce a transient ROS-signal

K. Zarse<sup>1</sup>, S. Schmeißer<sup>1,2</sup>, M. Groth<sup>3</sup>, S. Priebe<sup>4</sup>, G. Beuster<sup>1</sup>, D. Kuhlow<sup>1,5</sup>, R. Guthke<sup>4</sup>, M. Platzer<sup>3</sup>, C.R. Kahn<sup>6</sup>, M. Ristow<sup>1,5</sup>;

<sup>1</sup>Department of Human Nutrition, Friedrich-Schiller-University, Jena,

<sup>2</sup>Leibniz Institute for Age Research, Fritz-Lipmann-Institute, Jena, <sup>3</sup>Genome

Analysis Group, Leibniz Institute for Age Research, Fritz-Lipmann-Institute,

Jena, <sup>4</sup>Systems Biology and Bioinformatics Group, Hans-Knöll-Institute,

Jena, <sup>5</sup>Dept. of Clinical Nutrition, German Institute of Human Nutrition

Potsdam-Rehbrücke, Nuthetal, Germany, <sup>6</sup>Section on Integrative Physiology

and Metabolism, Joslin Diabetes Center, Harvard Medical School, Boston, USA.

**Background and aims:** In mammals, impaired insulin like growth-factor 1 and insulin signaling (iIS) is linked to several chronic diseases, including type 2 diabetes, cancer and neurodegeneration, whereas in the well-characterized model organism *C. elegans*, reduced iIS due to impaired expression of daf-2 (mammalian insulin-/IGF-1-receptors orthologue), extends life expectancy by more than two-fold. In the present study, we address the hypothesis that iIS induces oxidative nonglucose metabolism and generates a reactive oxygen species (ROS) imbalance which in turn is instrumental for the life span-extending capabilities of iIS in *C. elegans*.

**Materials and methods:** We analyzed three different models of iIS: a (I) *C. elegans* strain carrying a mutant daf-2(e1370) gene, as well as mammalian fibroblasts either (II) homozygously deficient for the insulin receptor substrate 1 (IRS1) or (III) heterozygously deficient for the insulin receptor.

**Results:** We show here that chronically iIS increases mitochondrial activity, but also unexpectedly reduces ROS levels. By contrast, RNAi-mediated knockdown of daf-2 in adult *C. elegans* not only impairs glucose metabolism, but also permanently increases mitochondrial activity and transiently increases ROS levels. Consistent with the concept of mitochondrial hormesis (mitohormesis), this latter ROS increase causes an adaptive response by inducing the activities of endogenous ROS-defense enzymes, superoxide dismutase and catalase, which culminates in increased stress resistance and ultimately reduces ROS levels despite constitutively increased mitochondrial activity. Inhibition of this ROS signal by different exogenous antioxidants reduces the life-extending capability of daf-2 impairment by up to 60%. Generation of this transient ROS signal was found to depend on AAK-2 (AMPK analogue), while PMK-1 (p38 MAP kinase) and SKN-1 (NRF-2 analogue) are required for ROS sensing to exert the retrograde response to the acute knockdown of daf-2. Lastly, transcriptome analyses identified mitochondrial L-proline catabolism to be uniformly upregulated in all three models of iIS studied, and impairment of L-proline catabolism in nematodes reduces the life span extending capacity of iIS while L-proline supplementation extends *C. elegans* life span.

**Conclusion:** Taken together, our results indicate that impaired iIS causes oxidative low-glucose L-proline metabolism which employs ROS as signaling molecules for the adaptive induction of endogenous stress defense to promote longevity.

*Supported by: Part of the JenAge program funded by BMBF 0315581.*

### 591

#### Overexpression of the adiponectin receptor AdipoR1 in rat skeletal muscle amplifies local insulin sensitivity

M.E. Cleasby<sup>1,2</sup>, K.L. Hoehn<sup>3</sup>, S.A. Patel<sup>1</sup>, R.T. Lawrence<sup>3</sup>, L. Sawbridge<sup>1</sup>, N.A. Talbot<sup>1</sup>, N. Turner<sup>2</sup>, G.J. Cooney<sup>2</sup>, J.P. Whitehead<sup>4</sup>, E.W. Kraegen<sup>2</sup>;

<sup>1</sup>Comparative Biomedical Sciences, Royal Veterinary College, London, UK,

<sup>2</sup>Diabetes and Obesity Program, Garvan Institute of Medical Research,

Sydney, Australia, <sup>3</sup>Department of Pharmacology, University of Virginia

Health System, Charlottesville, USA, <sup>4</sup>Metabolic Medicine, Mater Medical

Research Institute, Brisbane, Australia.

**Background and aims:** Adiponectin is a multimeric adipokine whose plasma levels are reduced in insulin resistant or obese humans or animals. Its effects are mediated through binding to receptors, of which adiponectin receptor 1 (AdipoR1) is the most abundant in skeletal muscle. Adiponectin has direct effects on rodent skeletal muscle strips to enhance glucose disposal and mitochondrial oxidation, and these effects are impaired in strips from high fat diet (HFD)-fed animals. However, the *in vivo* significance of altered adiponectin sensitivity and the molecular mechanisms of the putative muscle insulin sensitising action of adiponectin have not been fully established.

**Materials and methods:** *In vivo* electrotransfer was used to overexpress AdipoR1 in single *tibialis cranialis* (TC) muscles of otherwise untreated rats and also in six week chow or HFD-fed rats which were subsequently subjected to hyperinsulinaemic-euglycaemic clamp. After one week, the effects on glucose disposal (radioactive tracer methodology), intracellular signalling (western blotting) and sphingolipid metabolism (GC-MS and qPCR) were investigated in test versus paired control muscles (n=6–15). Data were analysed using paired t-test or two-way ANOVA with repeated measures, as appropriate.

**Results:** AdipoR1 was overexpressed 5.5-fold ( $p<0.001$ ) in basal rat TC muscles, which resulted in a 17% increase in glycogen content ( $p=0.011$ ) and increased phosphorylation of IRS1 (Tyr612, 41%,  $p=0.006$ ), Akt (Ser473, 34%,  $p=0.003$ ) and GSK3 $\beta$  (Ser9, 21%,  $p=0.030$ ). AdipoR1 was overexpressed ~3-fold in both clamped chow and HFD-fed rats, which resulted in increased insulin-stimulated glucose uptake ( $p=0.039$ ), glycogen synthesis ( $p=0.009$ ) and glycogen content ( $p<0.001$ ) in muscles of both feeding groups, implying local amelioration of the HFD-induced defect in glucose disposal. This was associated with increased phosphorylation of IRS1 ( $p<0.001$ ), Akt ( $p=0.015$ ) and GSK3 $\beta$  ( $p=0.004$ ). AdipoR1 overexpression (OE) also caused increased phosphorylation (activation) of AMP-activated protein kinase (pT172,  $p<0.001$ ) and its target acetyl coA carboxylase (pS79,  $p=0.050$ ), as well as increased protein levels of PGC1 $\alpha$  ( $p<0.001$ ) and UCP3 ( $p<0.001$ ), indicative of increased mitochondrial biogenesis and oxidation. Although neither HFD-feeding nor AdipoR1 OE caused generalised changes in sphingolipids, including ceramides, AdipoR1 OE did reduce levels of sphingosine 1-phosphate ( $p=0.011$ ), ceramide 18:1 ( $p=0.020$ ), ceramide 20:2 ( $p=0.031$ ) and dihydroceramide 20:0 ( $p=0.036$ ), plus mRNA levels of the ceramide synthetic enzymes ceramide synthase-1 ( $p=0.003$ ), serine palmitoyl transferase ( $p=0.022$ ) and sphingolipid delta-4 desaturase ( $p=0.027$ ).

**Conclusion:** This study demonstrates that therapeutic targeting of improved adiponectin sensitivity may be beneficial in the treatment of IR in muscle. The insulin sensitising effect of adiponectin in muscle involves an additive effect on PI3-kinase signalling and enhanced mitochondrial function, which may be mediated through selective reductions in sphingolipid species.

Supported by: The Wellcome Trust, the Australian NH&MRC, Diabetes UK

## 592

### Dissociation between *in vivo* and *in vitro* muscle mitochondrial function in patients with type 2 diabetes

C. Brøns<sup>1</sup>, B. Mortensen<sup>2</sup>, K. Pilgaard<sup>2</sup>, K. Rasmussen<sup>1</sup>, P. Poulsen<sup>3</sup>, A. Vaag<sup>1</sup>, B. Quistorff<sup>4</sup>

<sup>1</sup>Rigshospitalet, Copenhagen, <sup>2</sup>Steno Diabetes Center, Gentofte, <sup>3</sup>Novo Nordisk A/S, Bagsvaerd, <sup>4</sup>University of Copenhagen, Denmark.

**Background and aims:** The role of mitochondrial dysfunction in the development of muscle insulin resistance in Type 2 diabetes (T2D) is controversial, and may depend on whether the mitochondrial function is determined *in vivo* or under *in vitro* conditions. In this study, we aimed to measure and compare *in vivo* and *in vitro* mitochondrial function in T2D subjects and in a control group of normoglycaemic healthy subjects.

**Materials and methods:** Twelve middle-aged, obese men with T2D (mean age  $54.2 \pm 1.14$  years, BMI  $31.4 \pm 1.37$  kg/m<sup>2</sup>) and 8 matched non-diabetic control subjects (mean age  $55.3 \pm 2.51$  years, BMI  $29.4 \pm 1.06$  kg/m<sup>2</sup>) participated in the study. The T2D patients were instructed to discontinue all medication 24 hours prior to the investigation. A standard OGTT was performed, and mitochondrial function was assessed *in vivo* by <sup>31</sup>P Magnetic Resonance Spectroscopy in the wrist/finger flexor and in the tibialis anterior muscles at rest and during recovery after an acute exercise bout. *In vitro* mitochondrial function was measured by respirometry in permeabilized muscle fibers from the tibialis anterior and quadriceps muscles. Statistical analysis was performed using paired and unpaired t-test and Fischers exact test when appropriate. Differences were considered to be statistically significant at a two-sided P-value less than 0.05.

**Results:** The T2D subjects had a higher HbA1c ( $P=0.01$ ) and an increased fasting plasma glucose level ( $P<0.01$ ) compared to the control subjects. The T2D subjects were comparable with respect to age, BMI and standard anthropometric and biochemical analyses. No difference was found between T2D subjects and controls in any measures of *in vivo* mitochondrial function during rest or recovery after acute exercise. However, T2D patients displayed decreased *in vitro* mitochondrial state 3 as well as state 4 respiration ( $P<0.05$ ) in both muscle fibre types compared with the controls. No correlations were found between *in vivo* and *in vitro* measurements of mitochondrial function in T2D subjects or controls.

**Conclusion:** Compensatory mechanisms including increased substrate availability during *in vivo* hyperglycaemic conditions may explain the dissociation between *in vivo* and *in vitro* mitochondrial function in T2D patients compared with controls. Whether impaired *in vitro* mitochondrial function may represent a primary metabolic defect, involved in the development of insulin resistance, or is a protective and thus compensatory down-regulation in T2D subjects due to increased substrate availability remains unknown.

Supported by: The Strategic Research Council, The EU 6th Framework EXGENESIS grant

## 593

### The mitochondrial target of thiazolidinediones (mTOT): insulin sensitisation through a mitochondrial metabolic switch

W.G. McDonald<sup>1</sup>, G.S. Cavey<sup>2</sup>, S.L. Cole<sup>1</sup>, D.D. Holewa<sup>1</sup>, A.S. Brightwell-Conrad<sup>1</sup>, R.F. Kletzian<sup>1</sup>, J.R. Colca<sup>1</sup>

<sup>1</sup>Metabolic Solutions Development Co., Kalamazoo, <sup>2</sup>Southwest Michigan Innovation Center Core Life Science Lab, Kalamazoo, USA.

**Background and aims:** The thiazolidinediones (TZDs) are insulin sensitizing agents of proven utility in the clinic but their use is compromised by side effects that are driven by PPAR $\gamma$ . A complete understanding of the mechanism of action of these compounds will allow for the development of more useful insulin sensitizers.

**Materials and methods:** We have used a TZD based photoaffinity probe coupled with mass spectrometry-based proteomics to identify a mitochondrial target of insulin sensitizing agents. To determine the degree to which this target is involved in the actions of insulin sensitizers, we have studied the ability of various analogs, including PPAR $\gamma$ -independent TZDs, MSDC-0160 and MSDC-0602, to enhance differentiation of brown adipose tissue (BAT) progenitor cells from genetically modified mice.

**Results:** The identified mitochondrial target (mitochondrial Target of TZDs, mTOT) is part of a phylogenetically conserved complex in the inner mitochondrial membrane, which is responsible for binding of these agents to the mitochondrion. The expression of mTOT is tightly regulated with the expression of another member of the complex, the mTOT-Like protein. Emerging work in yeast and *Drosophila* has suggested that this protein complex is involved with transport of pyruvate into the matrix of the mitochondria. UK5099, a specific pyruvate transport inhibitor, potentially blocks the photoaffinity crosslinking of mTOT. Ablation of the gene encoding mTOT results in embryonic lethality in mice (E11, E12). A targeted mutation that deletes the first 16 amino acids of the amino terminal sequence results in viable animals, however, expression of the mTOT-Like protein is severely depressed in some tissues. Progenitor cells isolated from the axillary adipose tissue from these animals were unable to differentiate in response to TZDs, perhaps due to low expression of mTOT-Like. Additionally, TZD drug action in BAT progenitor cells causes post-translational modifications such as dephosphorylation of mitochondrially associated GSK3 $\beta$ , as well as changes in the lysine acetylation state of a number of mitochondrial proteins.

**Conclusion:** The identified mitochondrial complex, containing both mTOT and mTOT-Like, appears to be involved in the metabolism of pyruvate, which places it at the crossroads of metabolic regulation. Identification of the mTOT target and understanding its role in metabolic signaling provides an alternative approach to discovery of novel, non-TZD insulin sensitizing agents.

## 594

### Muscle transcripts of mitochondrial oxidative metabolism genes associate with fuel selection, but do not respond to testosterone therapy in men with subnormal testosterone

S.J. Petersson, L. Frederiksen, M. Andersen, K. Højlund; Department of Endocrinology, Section for Molecular Diabetes & Metabolism, Odense University Hospital, and Institute of Clinical Research, University of Southern Denmark, Denmark.

**Background and aims:** An association between low serum testosterone levels, low insulin sensitivity and reduced mitochondrial function has previously been identified in men. We have shown that testosterone therapy promotes a shift in fuel selection towards increased lipid oxidation and decreased glucose oxidation as well as a reduction in plasma adiponectin. This study aims to investigate the relationship between muscle transcripts involved in mitochondrial oxidative metabolism and whole-body measurements of insulin sensi-

tivity, glucose and lipid oxidation, as well as the effect of testosterone therapy on these muscle transcripts in men with subnormal testosterone levels.

**Materials and methods:** Skeletal muscle biopsies were obtained from elderly men ( $n=25$ ) with subnormal bioavailable testosterone before and after treatment with either testosterone gel ( $n=12$ ) or placebo ( $n=13$ ) for 6 months. Insulin-stimulated values of glucose disposal (Rd) and estimates of glucose and lipid oxidation in the basal state were assessed by euglycemic hyperinsulinemic clamps combined with indirect calorimetry. Muscle RNA of a selected set of genes involved in oxidative phosphorylation (OXPHOS), tricarboxylic acid cycle (TCA cycle), lipid oxidation and transport, and adiponectin-AMPK signaling was extracted and reverse transcribed, and gene expression was examined by qRT-PCR. Significance levels:  $**p<0.01$  and  $*p<0.05$ .

**Results:** In the total cohort, Rd was not significantly associated with muscle transcripts involved in OXPHOS, TCA cycle or lipid oxidation, but correlated negatively with the AMPK subunit, *PRKAG3* ( $r=-0.56^{**}$ ) and positively with *CPT1B* ( $r=0.50^{*}$ ). Basal glucose oxidation correlated positively with muscle mRNA levels of *NDUFS1* ( $r=0.46^{*}$ ), *SDHA* ( $r=0.51^{**}$ ), *UQCRC1* ( $r=0.65^{**}$ ), *COX5B* ( $r=0.52^{**}$ ), *ATP5A1* ( $r=0.58^{**}$ ), *CS* ( $r=0.43^{*}$ ), *ETFA* ( $r=0.51^{**}$ ), *ACADVL* ( $r=0.45^{*}$ ), *HADH* ( $r=0.47^{*}$ ), and *PPARGC1A* ( $r=0.44^{*}$ ). Basal lipid oxidation showed an inverse relationship with the OXPHOS muscle transcripts *UQCRC1* ( $r=-0.47^{*}$ ), *COX5B* ( $r=-0.46^{*}$ ), *ATP5A1* ( $r=-0.43^{*}$ ), and *PPARGC1A* ( $r=-0.43^{*}$ ). Pretreatment levels of bioavailable testosterone correlated positively with several transcripts (*NDUFS1*, *UQCRC1*, *COX5B*, *ATP5A1* and *ACADVL*;  $r=0.43$ – $0.47$ , all $^{*}$ ). Testosterone therapy had no effect on muscle mRNA levels of genes involved in OXPHOS, TCA cycle or lipid metabolism despite a significant placebo-controlled increase in whole-body lipid oxidation. However, in the testosterone treated group, a decrease in plasma adiponectin was accompanied by down-regulation of *ADIPOR2* (1.2 fold;  $p=0.041$ ). The post-treatment mRNA levels of *PRKAG3* ( $p=0.039$ ) was lower in the testosterone treated group.

**Conclusion:** In elderly men with subnormal testosterone levels, muscle transcripts involved in OXPHOS, TCA cycle and lipid oxidation are not associated with insulin sensitivity, but rather with a high rate of glucose oxidation. However, the shift in fuel selection induced by testosterone treatment was not accompanied by changes in mitochondrial biogenesis, whereas the decrease in plasma adiponectin was accompanied by reduced mRNA levels of one of its receptors.

Clinical Trial Registration Number: NCT00700024

Supported by: The Novo Nordisk Foundation

## 595

### An mtDNA mutation in the cytochrome-c oxidase impacts age-related processes in liver and pancreatic islet

J. Niemann, L. Wuchert, J. Schultz, F. Nulle, M. Tiedge, S. Baltrusch, C. Johné; Institute of Medical Biochemistry and Molecular Biology, University Rostock, Germany.

**Background and aims:** The cellular energy homeostasis is crucially dependent upon coordinated function of the mitochondrial respiratory chain. Mutations accumulating during ageing in the mitochondrial genome (mtDNA) directly affect subunits of the respiratory chain complexes and, thus, their assembling and function. Recently, mtDNA mutations were discussed to contribute to the development of type 2 diabetes mellitus. Therefore the aim of this study was to investigate pancreatic islet and liver function in mice carrying a single mtDNA mutation in the cytochrome-c oxidase (complex IV) of the respiratory chain.

**Materials and methods:** Pancreatic islets and liver were isolated from C57BL/6J-mt<sup>NOD</sup> (mtNOD) and C57BL/6J (control) mice. Gene expression was analyzed by quantitative RT-PCR. Mitochondrial morphology was determined by MitoTracker staining. Basal and glucose-induced insulin secretion was analyzed by ELISA.

**Results:** The nt9348 mtDNA variant (mtNOD) resulted in an amino acid change from valine to isoleucine in the subunit 3 of the cytochrome-c oxidase. Cytochrome-c oxidase gene expression in the liver of the mtNOD mice was comparable to controls. At the age of 12 months gene expression of the antioxidative enzyme Cu/Zn SOD was significantly increased in liver of mtNOD and control mice indicating an age-dependent increase in generation of reactive oxygen species (ROS). The gene expression of the uncoupling protein UCP2 and the enzyme pyruvate carboxylase remained unchanged in the liver of 3, 6, and 12 month old control animals. In contrast, there was an age-dependent decrease in the UCP2 expression and a significant increase in the pyruvate carboxylase expression in the liver of mtNOD mice pointing out a positive mitochondrial adaption process. Aggregation and loss of the

homogenous mitochondrial structure was observed during ageing in the liver of mtNOD and control mice. However, in mtNOD mice significant changes of the mitochondrial network structure occurred already at early lifetime between the age of 3 to 6 months. Interestingly, a higher amount of loops and circular structures contributed to the mitochondrial network in hepatocytes from mtNOD mice. Islets from mtNOD mice showed slightly higher glucose-induced insulin secretion compared to controls. At the age of 3 and 6 month basal insulin secretion of mtNOD islets was significantly higher than in controls. In 12 month old animals the basal insulin secretion increased as well in the controls resulting in a comparable secretion.

**Conclusion:** Our data indicate that a single mtDNA mutation in the cytochrome-c oxidase significantly affected the mitochondrial network structure in liver. These morphological changes are partially linked to ROS generation and antioxidative response. The C57BL/6J-mt<sup>NOD</sup> mouse strain represents an interesting model to study trigger effects of age-dependent mitochondrial phenotypes in the pathogenesis of type 2 diabetes mellitus.

Supported by: BMBF

## 596

### Age-dependend effects of the ATP8 mutation on mitochondrial gene expression and energy metabolism in liver of conplastic B6-mt<sup>FVB</sup>

F. Nulle, J. Niemann, M. Tiedge, S. Baltrusch, H. Weiss; Insitute of Medical Biochemistry and Molecularbiology, University of Rostock, Germany.

**Background and aims:** The conplastic mouse strain B6-mt<sup>FVB</sup> (FVB) carries a mitochondrial DNA polymorphism in the Atp8 gene coding for the regulatory subunit 8 of the ATP-synthase (nt7778T Asp to Tyr). This mutation resulted in higher ROS production and a pathological mitochondrial phenotype in different tissues compared to the B6-mt<sup>AKR</sup> (AKR) control strain. In this study we investigated age-dependent effects of the Atp8 mutation on mitochondrial gene expression profile, expression of respiratory chain complexes and energy state in the liver.

**Materials and methods:** Liver samples were collected from FVB and AKR mice at the age of 3, 6, 9 and 12 months ( $n = 6$ ). ATP-content was measured by a luminometric assay. The expression of specific mitochondrial genes was quantified by RT-qPCR and RT-PCR Array analyses (Qiagen, Mouse Mitochondria). Protein expression of the respiratory chain complexes and ATP-synthase were quantified by Western Blot analysis.

**Results:** Feeding a standard diet AKR and FVB mice showed comparable age-dependent gain in body weight and normal blood glucose levels. After 3 months ATP-synthase protein expression was higher in liver from FVB mice compared to AKR controls. Protein expression of complex IV showed a significant decrease during aging in FVB and AKR ( $p<0.05$ ) mice. Both strains showed no differences in ATP-content of liver tissue until the age of 6 months. At the age of 9 months a significant 1.8-fold decrease of ATP-content was observed in liver from FVB mice ( $AKR 18 \pm 2 \mu\text{mol/g}$  vs.  $FVB 10 \pm 2.8 \mu\text{mol/g}$ ,  $p < 0.01$ ). At the age of 3 months a higher gene expression of the molecular chaperone aryl-hydrocarbon receptor-interacting protein (Aip) (7-fold), SOD1 (2.4-fold), SOD2 (1.6-fold), UCP2 (2.2-fold), Bcl-2 (1.9-fold), mTOR (2-fold) and ATP-synthase (1.5-fold;  $p<0.05$ ) was observed in liver from FVB mice compared to AKR controls. At the age of 6 months the expression of UCP2 (1.9-fold), mTOR (1.9-fold) and ATP-Synthase (1.7-fold;  $p<0.05$ ) remained higher in FVB compared with AKR mice. After 12 months FVB mice showed a further increase of Aip (8-fold), SOD1 (2.5-fold) and Bcl-2 (3.5-fold) gene expression. In contrast, expression of mitochondrial protein import complexes, namely the protein translocators Timm17a and Timm8a1 and solute carrier transport proteins (Slc25a13/Slc25a5: adenine nucleotide translocator, Slc25a22: glutamate carrier) were significantly down-regulated in the liver from FVB mice at the age of 12 months compared to 3 months.

**Conclusion:** The data indicate that FVB mice with the Atp8 mutation compensated generation of reactive oxygen species in the liver through up-regulation of antioxidative enzymes. In addition the increased expression of the apoptosis-inhibitor factor Bcl-2 during ageing preserved cell viability. Mitochondrial DNA mutations induce mitochondrial adaptation processes during adolescence to guarantee sufficient energy demands of cells. However, at high age compensatory mitochondrial dynamics limits substrate fluxes into the mitochondria with concomitantly lower ATP levels. Thus, conplastic mtDNA mouse strains are ideal models to identify gene signatures of mitochondrial dysfunction that determine susceptibility to metabolic stress and type 2 diabetes at different stages of lifetime.

Supported by: Federal ministry of education and research (BMBF), GERONTOSYS 2 program



## PS 039 Exercise: impact and benefits

597

### Epigenetic regulation of muscle plasticity in response to endurance exercise

T. Kanzleiter, H. Vogel, N. Hallahan, M. Jähnert, A. Schürmann; Dife, Nuthetal, Germany.

**Background and aims:** Skeletal muscle accounts for ~50% of energy expenditure and is a major tissue for nutrient oxidation. Muscle plays a key role in insulin-stimulated postprandial glucose disposal and is the main site of lipid oxidation in the body; both processes are known to be impaired in type 2 diabetes. Physical exercise is well known for its beneficial effects on muscle metabolism. Recently it was shown for a panel of five genes that acute exercise can modify DNA-methylation in human skeletal muscle. The aim of our study was to investigate changes in muscle DNA-methylation in response to endurance training of mice on a genome wide level to provide a comprehensive picture of the extent of epigenetic effects.

**Materials and methods:** C57BL/6 mice were exercised (EX) on a motorized treadmill (5 days/week) for 4 weeks with increasing training challenge or remained sedentary (SED). Quadriceps muscles were analysed on Agilent microarrays to provide genome wide data on changes in gene expression. Genome wide DNA-methylation patterns were identified with reduced representational bisulfite sequencing (RRBS). An overlay of differentially expressed and methylated genes was calculated to identify genes with changes in DNA-methylation that result in functional consequences. Candidate genes were verified by RT-PCR and gene specific bisulfite sequencing.

**Results:** A total of 3020 genes showed significant changes in muscle gene expression between the EX and SED group. RRBS identified 461 genes which had significant changes in their CpG-methylation in their putative promoter regions. The overlay revealed 63 genes which showed changes in DNA-methylation AND gene expression suggesting functional consequences of the changes in DNA-methylation. Pathway analysis of the candidate genes revealed pathways involved in muscle growth, inflammation and mitochondrial gene expression.

**Conclusion:** Our data provide compelling evidence that prolonged endurance exercise training results in differential DNA-methylation and expression of genes involved in remodelling of skeletal muscle. This suggests that epigenetic mechanisms contribute to muscle plasticity and might therefore lead to novel strategies of treating impaired muscle metabolism.

*Supported by: German Ministry of Education and Research (DZD and NEU-ROTARGET)*

598

### Effects of acute endurance exercise on gene expression in human skeletal muscle: search for putative myokines

M. Catoire<sup>1</sup>, M. Mensink<sup>1</sup>, P. Schrauwen<sup>2</sup>, S. Kersten<sup>1</sup>;

<sup>1</sup>Human Nutrition, Wageningen University, <sup>2</sup>Human Biology, Maastricht University, Netherlands.

**Introduction and aim:** Exercise is known to be a powerful way to prevent and treat type 2 diabetes mellitus. Skeletal muscle is the predominant organ impacted by exercise but many of the metabolic changes induced by exercise may involve the liver. An important question is how exercise- or better skeletal muscle contraction - can alter hepatic function and insulin action. It is hypothesized that myokines play an important role in the cross-talk between skeletal muscle and liver. Since only a few myokines are known so far, identifying unknown myokines might provide valuable information for future research on the pathogenesis and treatment of type 2 diabetes mellitus. The aim of this study was to investigate the effect of acute endurance exercise on gene expression in skeletal muscle, with a focus on potential myokines, and to assess the importance of local and systemic effects of exercise on gene expression in skeletal muscle.

**Methods:** Nine healthy, male subjects (40–60 years) cycled for one hour with one leg at a submaximal rate. The passive leg served as control. Before and after exercising, muscle biopsies were taken from both legs and analyzed with microarray to determine which genes changed in response to exercise. Venous blood samples were taken before, directly after and after 2 hours of recovery.

**Results:** One-legged cycling induced a significant change in 938 genes ( $p < 0.01$ ), of which 674 were significantly upregulated and 264 genes were

downregulated. In contrast, in the control leg only 516 genes changed significantly ( $p < 0.01$ ; 384 upregulated, 132 downregulated). There was an overlap of 285 genes between both legs. Secretome analysis revealed that in the exercising leg 73 genes code for potentially secreted proteins (i.e. CXCL2, CX3CL1, MCP-1, FGF6, VEGFA), among them none of the established myokines (IL-6, IL-8, IL-15, BDNF). Plasma levels of several established and putative myokines were measured and this revealed that ANGPTL4 and MCP-1 showed a significant increase after exercise (ANGPTL4;  $p < 0.05$ ) and after 3 hours of recovery (ANGPTL4, MCP-1;  $p < 0.01$ ). IL-6, IL-8, IL-15, BDNF and VEGFA did not increase during exercise or recovery.

**Conclusion:** Exercise-induced gene expression changes are induced by both systemic and local stimuli. Several putative myokines are identified, in literature linked to processes like angiogenesis, growth, insulin signaling and lipid metabolism. MCP-1 and ANGPTL4 are elevated also in plasma after exercise and therefore could play a role in the cross-talk between muscle and liver. Plasma levels of other putative myokines will be measured (CXCL2, CX3CL1).

*Clinical Trial Registration Number: NCT01316731*

*Supported by: Dutch Diabetes Foundation*

599

### The expression of miRNAs in human muscle tissue is influenced by low physical activity

M.M. Kristensen<sup>1</sup>, J. Bork-Jensen<sup>2</sup>, A. Vaag<sup>2</sup>, F. Dela<sup>1</sup>;

<sup>1</sup>Department of Biomedical Sciences, <sup>2</sup>Rigshospitalet, University of Copenhagen, Denmark.

**Background and aims:** MicroRNAs (miRNAs) are important cellular regulators of gene expression in both health and disease. These 19–25 nucleotide long RNA sequences are expressed endogenously in almost all animal and plant species. Studies have found changes in miRNA levels in muscle and blood in response to training. Elucidating the roles of miRNAs in physically inactive humans is important to understand how sedentarism influences gene expression and potentially insulin resistance. We examined the influence of nine days of bed rest on miRNA expression profiles in human skeletal muscle tissue.

**Materials and methods:** 20 healthy young males were studied before and after bed rest for nine days and after four weeks of retraining. Muscle biopsies (vastus lateralis) were used for RNA extraction and miRNA expression profiling was performed using LNA arrays (Exiqon, Denmark). Linear models for microarray analysis (limma) was used to detect differentially expressed miRNAs using Benjamini and Hochberg's false discovery rate (fdr) adjustment for multiple testing. *In silico* target predictions (Targetscan) were performed to understand the biological implications further.

**Results:** Comparing microarray miRNA expression profiles before and after bed rest, we identified seven differentially expressed miRNAs ( $P_{(fdr)} \leq 0.1$ ) of which three were down-regulated (miR-486-3p, miR-24-2\*, and miR-126) and four were up-regulated (ebv-miR-BART2-5p, miR-1246, miR-21, and miR-1908) after bed rest. Interestingly, none of the miRNAs returned to pre-bed rest levels after the retraining period. Targetscan revealed multiple possible targets for each of the seven differentially expressed miRNAs. Among these were *IRS-1* (insulin receptor substrate 1) being a target of miR-126 as well as *PIK3R1* (phosphoinositide-3-kinase, regulatory subunit 1) being a target of miR-21.

**Conclusion:** We identified seven miRNAs with altered expression levels in response to short term bed rest, and none of them returned to the pre-bed rest values even after four weeks of retraining. Predicted targets of the miRNAs relevant to understanding insulin resistance included *IRS-1* and *PIK3R1*. Ongoing analyses should support these findings and illuminate the relation between these miRNAs and their respective targets. In conclusion, nine days of inactivity induced changes in the expression of miRNAs with relevance to insulin resistance, which in turn were not reversed by four weeks of retraining.

*Supported by: The Faculty of Health Sciences, University of Copenhagen*

## 600

**The effects and physiological mechanisms of free-living interval-walking training on glycaemic control in type 2 diabetes patients: a randomised, controlled trial**K. Karstoft<sup>1</sup>, K. Winding<sup>1</sup>, S.H. Knudsen<sup>1</sup>, B.K. Pedersen<sup>1</sup>, J.S. Nielsen<sup>2</sup>, T.P. Solomon<sup>1</sup>;<sup>1</sup>Faculty of Health Sciences, University of Copenhagen, Rigshospitalet,<sup>2</sup>Department of Endocrinology, Diabetes Research Centre, Odense University Hospital, Denmark.

**Background and aims:** In type 2 diabetes patients, free-living walking training is feasible but shows limited effect upon glycaemic control variables. On the other hand, interval training methods have shown huge improvements in glycaemic control but suffer from lower adherence rates. In this study, we first evaluated the feasibility of free-living walking training in type 2 diabetes patients; secondly, we investigated the effects of interval-walking versus continuous-walking training upon glycaemic control; and thirdly, we assessed the underlying physiological mechanisms of changes in glycaemic control.

**Materials and methods:** Subjects with type 2 diabetes ( $58.7 \pm 1.4$  years,  $29.5 \pm 0.9$  kg/m<sup>2</sup>) were randomized to a control group ( $n=8$ ), a continuous-walking training group ( $n=12$ ), or an interval-walking training group ( $n=12$ ). Training groups were instructed to train 5 sessions per week, 60 minutes per session and were controlled with an accelerometer and a heart rate monitor. Before and after the 4 month intervention, maximal oxygen consumption (VO<sub>2max</sub>) was assessed, glycaemic control was measured using continuous glucose monitoring (CGM), and insulin secretion/sensitivity was measured using a hyperglycaemic clamp ( $5.4$  mmol/l above fasting glucose concentration).

**Results:** Training groups demonstrated high and equal training adherence ( $89 \pm 4\%$ ), and training energy-expenditure and mean training intensity were comparable. VO<sub>2max</sub> was unchanged in the control group and continuous-walking group, but increased in the interval-walking group ( $16 \pm 4\%$ ,  $P<0.05$ ). Glycaemic control (mean CGM glucose levels) worsened in the control group (delta mean CGM glucose =  $1.2 \pm 0.4$  mmol/l,  $P<0.05$ ), whereas mean and maximum CGM glucose levels decreased in the interval-walking training group (delta mean CGM glucose =  $-0.8 \pm 0.3$  mmol/l,  $P=0.05$ , delta maximum CGM glucose =  $-2.8 \pm 0.8$  mmol/l,  $P<0.05$ ). The continuous-walking training group showed no changes in glycaemic control. In the interval walking training group, the insulin sensitivity ( $57 \pm 17\%$ ,  $P<0.05$ ) increased, whereas the insulin secretion did not change ( $3 \pm 6\%$ ,  $P>0.05$ ). The disposition index increased comparable to the insulin sensitivity ( $60 \pm 16\%$ ,  $P<0.05$ ). In the continuous-walking and control group, no changes were seen in any of these parameters.

**Conclusion:** Free-living walking training is feasible in type 2 diabetes patients and interval-walking training is superior to energy-expenditure matched continuous-walking training upon improving glycaemic control. Furthermore, interval-walking induced improvements in glycaemic control seem to be dependent on improvements in insulin sensitivity and increased disposition.

Clinical Trial Registration Number: NCT01234155

Supported by: DD2, Danish Agency for Science, Trygffonden, EFSD/Amylin grant

## 601

**Effects of high-intensity interval training on glucose and fat metabolism in healthy, sedentary middle aged men**A.M. Savolainen<sup>1</sup>, K.K. Kalliokoski<sup>1</sup>, J.J. Eskelinen<sup>1</sup>, V. Lepomäki<sup>1</sup>, I. Heinonen<sup>1</sup>, K. Virtanen<sup>1</sup>, R. Parkkola<sup>1</sup>, J. Kapanen<sup>2</sup>, J. Knuuti<sup>1</sup>, P. Nuutila<sup>1</sup>, J.C. Hannukainen<sup>1</sup>;<sup>1</sup>Turku PET Centre, University of Turku, Finland, <sup>2</sup>Paavo Nurmi Centre, Turku, Finland.

**Background and aims:** Lifestyle interventions have been shown to improve insulin sensitivity in liver and abdominal adipose tissues in obese patients and in patients with prediabetes or type 2 diabetes mellitus. Recently, two weeks of low-volume, high-intensity interval training (HIT) has been shown to increase whole body insulin sensitivity and glucose metabolism in skeletal muscle. The aim of this study was to investigate whether HIT also affects glucose and fatty acid metabolism in internal organs.

**Materials and methods:** Eight healthy, sedentary, middle aged men (mean  $\pm$  SD, age:  $47 \pm 5$  years; BMI:  $26 \pm 2.9$  kg·m<sup>-2</sup>; VO<sub>2max</sub>:  $34 \pm 4$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) were studied before and after two weeks and six sessions of HIT (4–6 x 30 s all out

sprints on a cycle ergometer with 4 minutes of recovery). Skeletal muscle, liver, pancreas, abdominal subcutaneous and visceral fat tissue insulin stimulated glucose uptake and fasting free fatty acid uptake were measured using FDG and FTHA PET -methods. In addition, muscle, liver and pancreas fat content was assessed with magnetic resonance spectroscopy.

**Results:** Following HIT intervention, VO<sub>2max</sub> increased by 4.7 % (from  $34 \pm 4$  to  $35.5 \pm 4$  ml·kg<sup>-1</sup>·min<sup>-1</sup>, student paired t-test,  $p=0.019$ ). Fasting serum free fatty acid concentration (from  $0.46 \pm 0.14$  to  $0.33 \pm 0.09$  mmol·l<sup>-1</sup>,  $p=0.054$ ) and plasma total cholesterol level (from  $5.4 \pm 0.7$  to  $4.5 \pm 0.6$  mmol·l<sup>-1</sup>,  $p<0.001$ ) decreased. Whole body insulin sensitivity increased by 12 % but without statistical significance ( $39.1 \pm 11.4$  vs.  $43.4 \pm 16.3$  μmol·kg<sup>-1</sup>·min<sup>-1</sup>,  $p=0.22$ ). Although glucose uptake in m. quadriceps femoris increased by 38% (from  $44 \pm 11$  to  $60 \pm 18$  μmol·kg<sup>-1</sup>·min<sup>-1</sup>,  $p=0.004$ ), two weeks of HIT had no influence on glucose uptake in liver, pancreas and abdominal adipose tissues. The results of free fatty acid uptake and MRS studies will be presented in the congress.

**Conclusion:** Two weeks of low-volume high-intensity interval training seems to be an effective method to improve insulin sensitivity in skeletal muscle, but has no effect on glucose metabolism in internal organs in healthy middle aged men. Further studies are needed in patients with prediabetes and type 2 diabetes to understand the role of exercise training in the metabolism of internal organs.

Clinical Trial Registration Number: NCT01344928

Supported by: EFSD/ Novo Nordisk, Ministry of Education, Academy of Finland, Orion Farmos

## 602

**Altered response to diet and exercise intervention in early-onset type 2 diabetes**D.J. O'Hanlon<sup>1</sup>, K. Wanic<sup>2</sup>, A. Pazderska<sup>2</sup>, S. Shah<sup>2</sup>, D.E. Cooper<sup>1</sup>, N. Collura<sup>2</sup>, D.J. O'Gorman<sup>1</sup>, J.J. Nolan<sup>3</sup>;<sup>1</sup>School of Health and Human Performance, Dublin City University, Ireland,<sup>2</sup>Metabolic Research Unit, St James's Hospital, Trinity College Dublin,Ireland, <sup>3</sup>Steno Diabetes Centre, Gentofte, Denmark.

**Background and aims:** We have reported that patients with early-onset type 2 diabetes (YT2) are much more insulin resistant, and less responsive to lifestyle and medical interventions than patients with later onset diabetes (OT2). We have found (unpublished data) that YT2 has a different metabolic signature, with higher fasting concentrations of total and several individual fatty acid species than OT2. The aim of this study was to further examine and compare the adaptive responses to lifestyle intervention in YT2 and OT2 subjects.

**Materials and methods:** YT2 and OT2 subjects were recruited for baseline examination, and to participate in a 6 month lifestyle intervention including a reduced-calorie diet ( $-2500$  kcal/week) and exercise training at 70% VO<sub>2max</sub>. Testing included a VO<sub>2max</sub> test to exhaustion, fasting anthropometric measurements (weight, waist circumference, and fat mass using bioelectrical impedance), a muscle biopsy from the vastus lateralis (to examine intrinsic mitochondrial function: state 3 respiration measured by respirometry) blood sampling to examine lipid profile, and an OGTT to measure insulin sensitivity (based on a 2 hour OGTT).

**Results:** We recruited 69 patients for evaluation: 23 YT2 ( $27.9 \pm 0.9$  years) and 46 BMI-matched OT2 patients ( $55.3 \pm 1.2$  years). At baseline, there were no differences between groups in waist circumference, VO<sub>2max</sub>, intrinsic mitochondrial function, or indices of insulin sensitivity. There was no difference in fasting triglyceride concentration, but despite a similar diet, the YT2 patients had higher total cholesterol ( $4.7 \pm 0.2$  vs  $4.2 \pm 0.1$  mmol/l,  $p=0.02$ ), and LDL-cholesterol ( $2.8 \pm 0.2$  vs  $2.2 \pm 0.2$  mmol/l,  $p=0.01$ ). A subgroup of 25 subjects (10 YT2 and 15 OT2) completed the lifestyle intervention, after which only the OT2 group had a significant reduction in fat mass ( $\Delta 3.1 \pm 0.9$  kg,  $p=0.01$ ). Both groups had comparable improvements in VO<sub>2max</sub>. However, a reduction in fasting triglyceride concentration ( $\Delta 0.37 \pm 0.1$  mmol/l,  $p=0.01$ ), and an increase in insulin sensitivity (OGIS-2hr:  $\Delta 47.5 \pm 18.3$  ml/min/m<sup>2</sup>,  $p=0.02$ ), occurred only in the OT2 group.

**Conclusion:** YT2 responds differently to lifestyle intervention than OT2. Despite equal compliance, only OT2 subjects exhibited a reduction in fat mass, with improvements in fasting triglyceride concentration and insulin sensitivity. These observations suggest that metabolic factors contribute to treatment resistance in YT2 patients.

Supported by: EFSD/Novo Nordisk grant

## 603

**Oxidative stress and pro-inflammatory proteins are involved in microalbuminuria after prolonged exercise in normoalbuminuric and normotensive type 1 diabetic runners**M. Rodríguez<sup>1,2</sup>, J. Cejas<sup>2</sup>, M. Cruzado<sup>2</sup>, J. Rodríguez Vitoria<sup>3</sup>, G. Minuchín<sup>3</sup>, G. Esteves<sup>3</sup>, C. Castro<sup>2</sup>;<sup>1</sup>University Hospital, <sup>2</sup>Area of Biological Chemistry, <sup>3</sup>Institute of Endocrinology, Metabolism and Nutrition, National University of Cuyo, Mendoza, Argentina.

**Background and aims:** Oxidative stress and inflammatory mediators are possible mechanisms of increased urinary albumin excretion (UAE) in type 1 diabetes (DM1). Prolonged exercise can increase UAE in diabetics and non-diabetics in a transitory way. It is not known the precise mechanisms of increased UAE in intense exercise and it is unclear whether the administration of antioxidants may have some effect.

**Materials and methods:** We evaluated in 12 runners 6 normoalbuminuric and normotensive DM1 and 6 non DM1 controls (C), total antioxidant state (TAS), monocyte chemoattractant protein (MCP-1) and ultra sensitive C-reactive protein (HS-CRP) before, at the end and 24 hours after finishing a first half marathon of 21km (1st M) and in a second half marathon (2nd M) after receiving vitamins E and C 1 g/d/7 days. Data (mean  $\pm$  SEM) were analyzed with ANOVA and Bonferroni post test.

**Results:** Basal UAE (mg/g) was normal in DM1 and C ( $5.66 \pm 1.2$  vs  $5.16 \pm 2.0$  mg/g  $P$ =ns). Immediately post 1st M there was a significant increase in UAE in both groups, higher in DM1 ( $65.1 \pm 20.7$  vs  $20.0 \pm 2.4$  mg/g  $P$ <0.01) and 24h after values returned to baseline. In the 2nd M vitamin supplement did not alter the behavior of UAE. After the 1st M TAS (mM/L) decreased in both groups ( $P$ <0.01 vs basal) with greater reduction in DM1 ( $0.6 \pm 0.1$  vs  $1.1 \pm 0.1$   $P$ <0.02), however vitamin intake was able to keep the TAS values up to the end of the race and 24h after. We then measure pro-inflammatory proteins. In the 1stM HS-CRP increased after 24h race in both groups, being higher in DM1 ( $5.5$  mg/dL  $\pm 0.9$  vs  $1.7 \pm 0.3$ ). MCP-1 aroused immediately finished the marathon in both groups ( $985.1$  pg/mL  $\pm 107.5$  vs  $710.9 \pm 77.5$ ) and 24h after returned to basal only in control groups. In the 2ndM both pro-inflammatory proteins behaved as in the 1stM.

**Conclusion:** The chronic hyperglycemic state with low antioxidant capacity and augmented MCP-1 and HS-CRP could be involved in higher UAE induced by sustained intense exercise in normoalbuminuric and normotensive DM1. Intake of vitamins E and C improves total antioxidant reserve but does not change UAE or inflammatory markers studied.

Supported by: FCM UNCuyo

## 604

**Continuous glucose monitoring in 42 runners with type 1 diabetes during an 18 km distance run in Brazil**R.N. Lamounier<sup>1,2</sup>, W.P. Valadares<sup>1</sup>, G.L.C. Mendes<sup>1</sup>, L.H.L. Cordeiro<sup>1</sup>, A.S. Silva<sup>1</sup>, M.G. Silva<sup>3</sup>, J.M. Couto<sup>1</sup>, C.C. Faria<sup>4</sup>, M.L. Miranda<sup>4</sup>, D.F. Motta<sup>5</sup>, D. Giannella-Neto<sup>6</sup>;<sup>1</sup>Endocrinology, Centro de Diabetes de Belo Horizonte, <sup>2</sup>Endocrinology and Diabetes, Hospital Mater Dei, Belo Horizonte, <sup>3</sup>Cardiology, Centro de Diabetes de Belo Horizonte, <sup>4</sup>Nutrition, Centro de Diabetes de Belo Horizonte, <sup>5</sup>Physiology, University of Minas Gerais (UFMG), Belo Horizonte, Brazil, <sup>6</sup>Gastroenterology, University of Sao Paulo (USP), Brazil.

**Background and aims:** Regular exercise is very important for type 1 diabetes mellitus (T1DM) patients. But can predispose to glucose variation and hypoglycemia. The project “Volta Monitorada de Belo Horizonte” aims to prepare subjects with T1DM to participate in long distance runs. We evaluated the glucose profile with continuous glucose monitoring (CGM), during an 18 km race.

**Materials and methods:** Forty-two subjects performed the race with CGM, in Belo Horizonte, Brazil. According to their treatment, they were classified in 3 groups: CSII ( $n=10$ ); Basal analogs (BA;  $n=22$ ); NPH ( $n=10$ ). All subjects were under intensive insulin regimens. Depending on their velocity in the race, they were divided in high (HP) or Low (LP) performers, if they were above or below the median velocity (MV) for the group. CGM readings were collected 24 h before and 36 h after the race. Capillary blood glucose (BG) was measured at the start, in the middle and at the end of the race. Glucose profile was assessed as a function of their performance and insulin treatment. Exposure to low and high glucose levels were assessed as well as glucose variability according to the CGM data, for the coefficient of variation (CV).

**Results:** Mean age was  $28.8 \pm 8.2$  years and time to finish the race was  $139 \pm 29.4$  min. MV was  $8.03$  (range  $5.48$ – $11.87$  km/h). Average HbA1c was  $7.84 \pm 1.34\%$  (similar between basal treatments). Subjects had been monitored for  $4333.2 \pm 108.8$  min, with  $866.64 \pm 339.25$  readings each one. Average CGM value was  $150.7 \pm 21.6$  mg/dL, similar between all 3 basal treatments as well as it was the exposure to hypo and hyperglycemia with  $32.4 \pm 37.6$  readings below  $54$  mg/dL and  $474.24 \pm 206.44$  readings above  $200$  mg/dL. When comparing BG after 9 km, in relation to the value at the start of the race, the reduction was significantly higher among CSII compared to BA ( $55.4\% \pm 25.4\%$  vs  $16.1\% \pm 46.1\%$ ;  $p=0.01$ ). HP (MV= $10.68$  km/h) subjects had lower glucose values compared to LP (MV= $6.80$  km/h); Median:  $140.7$  vs  $158.28$  mg/dL;  $p=0.03$ ; as well as lower glucose variation according to CV ( $0.39$  vs  $0.50$ ;  $p<0.01$ ). Those with HbA1c  $<7.0\%$  had a better performance than those with HbA1c  $\geq 7.0\%$  (Median:  $9.32$  vs  $7.34$  km/h;  $p<0.01$ ).

**Conclusion:** Among these runners, glucose variability was similar through different basal insulin regimens. The overall low exposure to hypoglycemia observed in this study (4% of the time below  $54$ mg/dL) could suggest a protective role of CGM for T1DM athletes. Interestingly, in the first 9 km the reduction in glucose was deeper in the CSII group, but throughout all the CGM period it was similar, considering the whole race and the CGM time before and after it. Individuals with better physical fit showed better glucose control and lower glucose variation according to CGM, which was in accordance with the observation that those with higher previous HbA1c, had worse athletic performance. This data suggests that among these T1DM runners, the better training was related to better glucose control, with less variability, independent of basal insulin regimen.

## 605

**Moderate exercise prevents morphological and functional adrenocortical alterations in rats with sucrose-induced insulin resistance**C. Martínez-Caleman<sup>1,2</sup>, J.M. Di Gruccio<sup>1,2</sup>, D. Jaume<sup>1,2</sup>, R. Sanchez<sup>1,2</sup>, M.E. Mercau<sup>1,2</sup>, F. Astort<sup>1,2</sup>, P. Arias<sup>3,4</sup>, C.B. Cymering<sup>1,2</sup>;<sup>1</sup>Human Biochemistry, University of Buenos Aires, <sup>2</sup>CEFyBO, Buenos Aires, <sup>3</sup>Physiology, University of Rosario, <sup>4</sup>Universidad Nacional del Litoral, Santa Fe, Argentina.

**Background and aims:** Previous results from our group demonstrate the impact of sucrose-induced insulin resistance on rat adrenocortical function and morphology. Physical activity reduces insulin resistance both in patients and in animal models with this metabolic derangement. Thus, we studied the effects of moderate physical exercise (PE) on the adrenocortical alterations already detected in rats with insulin resistance induced by sucrose-rich diets (SRD).

**Materials and methods:** Adult male Wistar rats received 30% sucrose in the drinking water or their usual diet for a period of 9 weeks. Sucrose-treated and control animals underwent a protocol of moderate PE on a treadmill (at  $0.8$  km/h up to  $6$  min,  $5$  days per week, groups SRD-E and C-E respectively). After 9 weeks, we measured insulin sensitivity (insulin tolerance test or ITT with  $0.75$  IU/kg insulin i.p.), adrenal function (basal and ACTH-stimulated serum corticosterone levels) and plasma ACTH levels. In adrenocortical tissue we determined triacylglyceride (TAG) and total cholesterol concentrations, the expression of proteins involved in steroidogenesis (StAR and CYP11A1) and its regulation (MC2R ACTH receptor), and inflammatory markers (TNF  $\alpha$ , F4/80, COX-2). We also performed a histochemical evaluation of the adrenal cortex. Similar analysis were performed also in sedentary animals receiving a SRD (SRD-S) or their usual diet (C-S). Values are expressed as mean  $\pm$  SEM. Differences between groups were analyzed by unpaired  $t$  test or by one-way ANOVA (Tukey's test).

**Results:** SRD-E animals exhibited lower body weight and glucose and insulin levels as compared to those measured in the corresponding SRD-S group. Results obtained by means of the ITT showed a significant amelioration of the  $k$  value (SRD-E  $1.88 \pm 0.43$  vs. SRD-S  $1.68 \pm 0.4$ ,  $\text{mg}^* \text{ml}/\text{min}$ ,  $p<0.005$ ). Insulin resistant animals submitted to PE showed significantly lower levels of adrenal TAG (SRD-E  $2.11 \pm 0.17$  vs. SRD-S  $2.89 \pm 0.19$   $\mu\text{g}^* \text{mg}$  tissue,  $p<0.01$ ). In agreement, the densitometric analysis of Sudan III staining of adrenocortical sections showed a significant decrease in lipid infiltration in SRD-E animals. PE also resulted in a significant decrease in the expression levels of CYP11A1, StAR, COX-2, F4/80 and TNF  $\alpha$ , and in elevated MC2R mRNA levels, as compared to the results obtained in the SRD-S group. Finally, PE blocked the increase in both basal ACTH (SRD-E  $48.7 \pm 5.56$  vs. SRD  $80.7 \pm 7.57$   $\text{pg}/\text{ml}$   $p<0.05$ ) and corticosterone (SRD-E  $7.8 \pm 0.11$  vs. SRD  $30.0 \pm 0.10$   $\text{ng}/\text{ml}$   $p<0.005$ ) levels present in SRD-S rats, and restored the subnormal adrenal



response to acute ACTH stimulation ( $\text{SRD-E } 4890 \pm 180$  vs.  $\text{SRD } 3840 \pm 131$  AUC arbitrary units  $p < 0.005$ ).

**Conclusion:** The implementation of a moderate PE protocol in SRD-treated animals normalized insulin sensitivity and prevented the onset of morphological and biochemical alterations in the adrenal cortex. This protocol also improved adrenal function and restored the response to acute ACTH stimulation. Taking these results, as well as previous data obtained using an insulin-sensitizing drug, in consideration, a causal relationship between insulin resistance, increased adrenocortical lipid deposition and deranged secretory function could be hypothesized.

*Supported by: PICT 2008 N°1034-UBACYT M021*

## PS 040 Aspects of carbohydrate metabolism

### 606

#### Altered glycolytic and gluconeogenic fluxes within the liver underlie the diurnal variation in glucose production in type 2 diabetes

J. Radziuk, S. Pye;

Medicine, The Ottawa Hospital, Canada.

**Background and aims:** In type 2 diabetes, endogenous glucose production (EGP) and gluconeogenesis display diurnal rhythms which drive the fasting hyperglycemia and are absent in weight-matched, healthy controls. The present study was designed to evaluate the intrahepatic metabolic dynamics underlying the altered pattern of glucose production in type 2 diabetes.

**Materials and methods:** Subjects with type 2 diabetes ( $n=9$ ) and age / BMI-matched normal controls ( $n=7$ ) who were fasting after breakfast (8:00h) underwent a further 24h fasting study starting at 14:00h. [ $\text{U-}^{13}\text{C}$ ]glucose and [ $3\text{-}^{14}\text{C}$ ]lactate were infused concomitantly. Acetaminophen was administered at 16:00h on the day the study was initiated and at 7:00h the following day. EGP and an index of gluconeogenesis were determined from the plasma tracer concentrations.  $\text{C}^{14}$  label in  $\text{C}_2\text{-C}_5$  of both plasma and glucuronide-derived glucose provided a measure of the degree of label randomization in circulating glucose and hepatic glucose-6-phosphate. [ $\text{U-}^{13}\text{C}$ ]glucose and  $\text{C}^{14}$  glucose specific activity were also determined in glucuronide glucose.

**Results:** Mirrored by the index of gluconeogenesis, EGP rose slowly overnight from  $8.4 \pm 0.6$  to  $11.2 \pm 0.4 \mu\text{mol kg}^{-1}\text{min}^{-1}$  ( $p < 0.05$ ) by 7:00h in diabetes before falling back to rates matching those in controls ( $8.2 \pm 0.2 \mu\text{mol kg}^{-1}\text{min}^{-1}$ ). The rising EGP increased plasma glucose concentrations to a morning peak of  $8.5 \pm 0.7 \text{ mmol/l}$  with a subsequent fall to  $6.0 \pm 0.3 \text{ mmol/l}$ . Control subjects remained near  $5.0 \pm 0.2 \text{ mmol/l}$ . After equilibration,  $\text{C}_2\text{-C}_5$  label rose from  $41 \pm 2$  in control to  $52 \pm 1\%$  ( $p < 0.05$ ) of total plasma glucose  $\text{C}^{14}$  label in diabetic subjects. Glucuronide  $\text{C}_2\text{-C}_5$  label however was the same at  $42 \pm 2$  and  $44 \pm 5\%$ . [ $\text{U-}^{13}\text{C}$ ] glucuronide glucose enrichment was  $14 \pm 2\%$  of plasma glucose in controls and  $29 \pm 3\%$  in diabetes ( $p < 0.05$ ), while  $\text{C}^{14}$ glucuronide specific activity (normalized to infusion rate of [ $3\text{-}^{14}\text{C}$ ]lactate) was  $1859 \pm 225$  and  $1006 \pm 82 \text{ dpm/mg}$  in control and diabetic subjects ( $p < 0.05$ ).

**Conclusion:** Taken together these data suggest, in type 2 diabetes, an increased randomization in circulating glucose of  $\text{C}^{14}$ label from the 3<sup>rd</sup> position of lactate. This is accompanied by a decrease of glucuronide  $\text{C}^{14}$  label overall in diabetes, in the face of cyclic increases in gluconeogenic flux to the circulation. The best explanation is increased rates of both glycolysis and gluconeogenesis within the liver in type 2 diabetes, possibly within different metabolic zones. An increased hepatic glucose uptake, tracked by the [ $\text{U-}^{13}\text{C}$ ] glucose tracer, appears to be entrained with the increased gluconeogenic flux. These altered fluxes underlie the diurnal variation in glucose production in diabetes.

*Supported by: CIHR and the Canadian Diabetes Association*

### 607

#### Estimated glucose disposal rate is associated with macro- and microvascular complications in adult type 1 diabetes patients

A. Florentiu, O. Savu, R. Barbu, D. Reurean-Pintilei, R. Lichiardopol, R. Rugina, E. Culita, M. Vintila;

<sup>1</sup>N.C. Paulescu Institute, Bucharest, Romania.

**Background and aims:** Insulin resistance in type 1 diabetes (T1DM) patients has been consistently linked to the prevalence of certain complications of diabetes and especially to cardiovascular disease (CVD). While the standard test for quantification of insulin resistance is the euglycemic hyperinsulinemic clamp, a simple formula for estimation of glucose disposal rate from clinical and metabolic parameters has been validated. In this study we aimed to assess the relation between estimated glucose disposal rate (eGDR) and prevalence of CVD, microvascular complications, and nonalcoholic fatty liver disease (NAFLD) in a group of adult T1DM patients.

**Materials and methods:** We assessed adult patients with T1DM, recording personal history of arterial hypertension (HTN), CVD, microvascular diabetic complications, NAFLD status, waist-to-hip ratio (WHR) and biochemistry (HbA1c). Resting electrocardiogram and ankle-brachial index were used to screen for unknown CVD. Fundoscopic eye examination, urine analysis and feet skin sensibility tests were used to screen for unknown microvascular dia-

betic complications. Abdominal ultrasonography was performed to diagnose incident NAFLD. eGDR was calculated according to the formula validated by Williams et al:  $eGDR = 24.31 - 12.22 \times WHR - 3.29 \times HTN - 0.57 \times HbA1c$  [mg/kg/min]. The prevalences of CVD, NAFLD and diabetic complications were analyzed according to tertiles of eGDR distribution. Logistic regression analysis was employed to define the relation between CVD, microvascular complications and NAFLD prevalence, and eGDR and other variables. SPSS software was used for statistical analyses. A  $p$  value  $<0.05$  was considered significant.

**Results:** Data from 267 patients (123 male, 144 female), aged between 18 and 78 years and with a mean (SD) disease duration of 13 (10.2) years, was collected. Patients with eGDR in the first tertile had an over 7-fold higher prevalence of CVD, as compared to the upper tertile (52.2% vs. 6.8%,  $p < 0.001$ ). We found no case of CVD in patients with  $eGDR > 8.2$  mg/kg/min. In logistic regression eGDR and CVD were correlated (adjusted  $r^2 = 0.18$ ,  $p < 0.001$ ). When age was introduced in the model the relation did not lose its statistical significance. A multiple logistic regression model including age and eGDR was the best in predicting CVD (adjusted  $r^2 = 0.26$ ). The microvascular diabetic complications (74.4% vs. 47.7%,  $p < 0.001$ ) and NAFLD (33.3% vs. 13.6%,  $p = 0.001$ ) were more prevalent in the first tertile of eGDR distribution, as compared to the third tertile.

**Conclusion:** Our data suggest that insulin resistance is associated with a greater prevalence of CVD in type 1 diabetic patients, independently of age and disease duration. The only other variable associated with CVD prevalence was age. Insulin resistance was also associated with the microvascular complications burden and the prevalence of NAFLD. Estimation of GDR by a simple formula can be useful in clinical practice to stratify the risk of macro and microvascular complications in selected type 1 diabetes patients.

## 608

### The insulin-independent glucose-induction of the hepatic gluconeogenic enzyme glucose 6-phosphatase involves distinct metabolic pathways and transcription factors

Z.H. Al-Oanzi<sup>1,2</sup>, J.L. Petrie<sup>1</sup>, S. Tudhope<sup>1</sup>, C. Arden<sup>1</sup>, L. Agius<sup>1</sup>;

<sup>1</sup>Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK, <sup>2</sup>College of Applied Medical Sciences, Al-Jouf University, Skaka, Saudi Arabia.

**Background and aims:** Elevated hepatic gluconeogenesis is a contributing factor to fasting and postprandial hyperglycaemia in type 2 diabetes. Elevated expression of glucose 6-phosphatase (G6pc) the enzyme that catalyses the final reaction in hepatic glucose production is generally attributed to a defect in insulin repression. However G6pc is also induced by hyperglycaemia. The aim of this study was to determine the mechanisms by which glucose induces G6pc in liver.

**Materials and methods:** Rat hepatocytes in short term primary culture were used to study the induction of G6pc mRNA expression by high glucose (25mM versus 5mM). The role of specific transcription factors was tested by over-expression of wild-type or dominant negative variants and by chromatin immunoprecipitation assays. The metabolic events by which glucose causes transcriptional activation were studied using selective inhibitors of the hexosamine pathway or expression of enzyme variants.

**Results:** Expression of G6pc mRNA in hepatocytes was repressed by 10 nM insulin but it was induced by glucose (25mM vs 5mM) in both the absence and presence of insulin (G6pc mRNA; 5mM, 1.0; 25mM,  $12 \pm 2.3$ ,  $P < 0.01$ ; 5mM+insulin,  $0.3 \pm 0.1$ ; 25mM+insulin,  $1.2 \pm 0.3$ ,  $P < 0.01$ ). The glucose-induction of G6pc expression was attenuated but not abolished by expression of a dominant-negative (DN) variant of Mlx and this attenuation was reversed by expression of ChREBP but not MondoA (25mM,  $8.9 \pm 2.7$ ; +Mlx-DN,  $4.4 \pm 1.4$ ,  $P < 0.05$ ; +Mlx-DN+ChREBP,  $14 \pm 3.2$ ,  $P < 0.05$ ; +Mlx-DN+MondoA,  $3.2 \pm 1.4$  NS). A role for endogenous ChREBP but not MondoA was further shown by chromatin immunoprecipitation assays, confirming an exclusive role for ChREBP-Mlx in the attenuation by Mlx-DN. Candidate transcription factors for the Mlx-independent component of the glucose-induction include Foxo transcription factors as confirmed by overexpression studies and lack of repression by Mlx-DN (25mM,  $3.8 \pm 1.3$ ; +Foxo3A,  $9.4 \pm 2.7$ ; +Mlx-DN,  $2.2 \pm 0.2$ ; +Mlx-DN+Foxo3A,  $5.8 \pm 0.6$ ). Two metabolic signals were identified in the G6pc-induction by high glucose. (i) Fructose 2,6-bisP, as supported by repression of G6pc induction with a Kinase-Deficient variant of PFKFBP1 that abolished the glucose elevation of this regulator (25mM,  $14 \pm 5.2$  vs 25mM+KD,  $3.7 \pm 1.1$ ); (ii) Hexosamine pathway flux as supported by the attenuation with a GFAT (glutamine:fructose 6-P amidotransferase) inhibitor (25mM,  $3.9 \pm 1.5$  vs 25mM+I,  $0.9 \pm 0.5$   $P < 0.05$ ). The independence of the two signals was shown by lack of effect of the GFAT inhibitor on fructose

2,6-bisP (5mM,  $34 \pm 7.1$ ; 25mM,  $281 \pm 46$  25mM+I,  $293 \pm 54$ ) and by the lack of effect of fructose 2,6-bisP depletion on hexosamine pathway end-product (5mM,  $0.7 \pm 0.6$ ; 25mM,  $1.1 \pm 0.7$  25mM+KD,  $1.0 \pm 0.6$ ).

**Conclusion:** The insulin-independent glucose-induction of G6pc in hepatocytes is in part mediated by ChREBP-Mlx and in part by additional transcriptional regulators. At least two independent metabolic signalling events involving fructose 2,6-bisP and flux through the hexosamine pathway are involved in the glucose induction. This supports the hypothesis that glucose induction of G6pc and the consequent glucose intolerance is a crucial adaptive mechanism to protect the liver from substrate overload that results in fatty liver.

Supported by: Medical research council, Diabetes UK and Al-Jouf university

## 609

### Postprandial capillary density is blunted in men with type 2 diabetes mellitus or metabolic syndrome

R.E. Van Genugten, E.H. Serné, D.H. Van Raalte, M. Diamant;

Diabetes Center, Department of Internal Medicine, VU University Medical Center, Amsterdam, Netherlands.

**Background and aims:** Microvascular capillaries are known to contribute to overall glucose uptake and a decreased functional capacity is related to peripheral insulin resistance. Steady-state hyperinsulinaemia increases capillary density (i.e. capillary recruitment), as measured by euglycaemic-hyperinsulinaemic clamp technique. We investigated whether similar responses occur after ingestion of a mixed meal and hypothesised that postprandial microvascular function, compared to healthy controls, is impaired in metabolic syndrome (MetS) and type 2 diabetes (T2DM), and is associated with insulin sensitivity and postprandial hyperglycaemia.

**Materials and methods:** We recruited 12 healthy men, 13 with MetS (IDF-criteria) without T2DM and 12 with uncomplicated T2DM. Microvascular function was assessed by skin capillary video microscopy. Capillary density at baseline and during post-occlusive peak reactive hyperaemia (PRH) was assessed fasting (T0), and 60 (T60) and 120 minutes (T120) after ingestion of a standardised solid mixed-meal (total 900 kCal/50g fat/75g carbohydrates/37g protein). Oral glucose insulin sensitivity (OGIS) and postprandial hyperglycaemia (4h-AUC<sub>glucose</sub>) were calculated. Repeated-measures ANOVA and Pearson correlation were used for statistical analysis.

**Results:** Groups were matched for age (mean $\pm$ SD:  $56.6 \pm 5.7$  y) and, as expected, differed significantly with respect to BMI, fasting and postprandial glucose, HbA1c and OGIS (all  $P < 0.05$ ). In the fasting state, baseline capillary density was similar among groups (controls  $40.1 \pm 6.6$ ; MetS  $35.5 \pm 7.9$ ; T2DM  $39.4 \pm 4.7$  n/mm<sup>2</sup>  $P = ns$ ), but PRH was lowest in T2DM, with intermediate values for MetS ( $P = 0.048$ ). Postprandial capillary recruitment was blunted in MetS and T2DM (PRH T60 vs. T0  $P < 0.05$  for HC only; T120 vs. T0  $P < 0.05$  for all groups). In all groups, postprandial AUC for PRH correlated with OGIS ( $r = 0.492$   $P < 0.05$ ) and with 4h-AUC<sub>glucose</sub> ( $r = 0.350$   $P < 0.05$ ).

**Conclusion:** Both MetS and T2DM individuals are characterised by decreased capillary density during PRH in the fasting state and impaired meal-related capillary recruitment. In pooled analysis, postprandial capillary recruitment during PRH was associated with both insulin sensitivity and postprandial hyperglycaemia. These findings suggest that in T2DM, microvascular dysfunction might contribute to postprandial hyperglycaemia while in individuals with MetS, who are at high risk to develop T2DM, it may contribute to glucose intolerance.

Clinical Trial Registration Number: NCT00721552

## 610

### In vivo insulin clearance regulation: a role for nitric oxide

F.O. Martins<sup>1,2</sup>, J.G. Jones<sup>1,3</sup>, R. Ribeiro<sup>2,3</sup>, A. Mari<sup>4</sup>, A. Natali<sup>2,5</sup>, M.P. Macedo<sup>2,3</sup>;

<sup>1</sup>Centre for Neurosciences and Cell Biology, University of Coimbra, Portugal, <sup>2</sup>Centro de Estudos de Doenças Crónicas, New University of Lisbon, Portugal, <sup>3</sup>Portuguese Diabetes Association (APDP-ERC), Lisbon, Portugal, <sup>4</sup>Institute of Biomedical Engineering, CNR, Padova, Italy, <sup>5</sup>Metabolism Unit, Department of Internal Medicine, University of Pisa, Italy.

**Background and aims:** Impairment on insulin clearance (IC) is an early event in the disturbance of glucose metabolism, as it is observed in first-degree relatives of type 2 diabetics and in obese insulin resistant subjects. IC is a major component of ineffective control of peripheral insulin levels found in

those conditions. The mechanisms that regulate IC have been overlooked but have a major influence on the amount of insulin that reaches the periphery. Even though it is well established that nitric oxide (NO) regulates peripheral insulin sensitivity and alters, *in vitro*, the activity of the insulin degrading enzyme (IDE), its role on IC and  $\beta$ -cell sensitivity modulation is not clear. Herein, we tested the hypothesis that, *in vivo*, NO decreases  $\beta$ -cell sensitivity and hepatic IC by inhibiting IDE activity.

**Material and methods:** In this study, 12 weeks male Wistar rats were divided in two groups: nitric oxide synthase (NOS) acutely inhibited [L-nitroarginine methyl ester (L-NAME); 35 ug/kg/min; i.v.] and NOS chronically inhibited (L-NAME 10 mg/kg; daily; 4 weeks; s.c.) animals. Each animal was submitted to an intra-enteric glucose tolerance test (IEGTT) and plasma samples (0, 15, 30, 60, 90 and 120 min after the glucose bolus) were collected for analysis of insulin and c-peptide levels. Hepatic IDE activity and expression were measured in liver tissue. Total IC was quantified from the ratio of plasma c-peptide and insulin levels as well as through the slope of the best linear fit between c-peptide and plasma insulin levels.  $\beta$ -cell sensitivity was obtained from the average slope of the lines in the representation of c-peptide plotted against the corresponding glucose levels.

**Results:** Acute but not chronic L-NAME treatment decreased plasma glucose excursions during the IEGTT (AUC: Control:  $21686 \pm 809,0$ ; Acute L-NAME:  $18356 \pm 511,9$ ,  $p < 0,05$ ; Chronic L-NAME:  $23233 \pm 499,6$ , n.s.). Both L-NAME treatments promoted an increase in total IC (AUC: Control:  $78237 \pm 3283$ ; Acute L-NAME:  $128278 \pm 7954$ ,  $p < 0,001$ ; Chronic L-NAME:  $103445 \pm 4344$ ,  $p < 0,05$ ) which was associated with an increase in hepatic IDE activity (RFUs/mg protein: Control:  $7,8 \pm 0,8$ ; Acute L-NAME:  $12,4 \pm 1,7$ ,  $p < 0,05$ ; Chronic L-NAME:  $10,4 \pm 0,4$ ,  $p < 0,05$ ). Hepatic IDE expression did not change with L-NAME treatment. Moreover, both acute and chronic L-NAME treatments also attenuated the decrease in IC occurring in the transition from fast to fed after the intraenteric bolus of glucose (% decrease: Control:  $66,9 \pm 4,5$ ; Acute L-NAME:  $49,2 \pm 4,4$ ,  $p < 0,05$ ; Chronic L-NAME:  $40,0 \pm 4,1$ ,  $p < 0,01$ ). Finally,  $\beta$ -cell sensitivity significantly increased with both acute and chronic L-NAME treatment (slope: Control:  $17,0 \pm 2,602$ ; Acute L-NAME:  $29,2 \pm 2,3$ ,  $p < 0,05$ ; Chronic L-NAME:  $10,24 \pm 3,0$ ,  $p < 0,05$ ).

**Conclusion:** In conclusion, our results support the hypothesis that NO is a physiological regulator of IC, by inhibiting hepatic IDE activity, and resulting in decreased  $\beta$ -cell sensitivity. These results support the hypothesis that in insulin resistance states, where inducible NOS is increased, IC is impaired and hyperinsulinemia come into sight.

Supported by: SFRH/BD/51194/2010 and PIC/IC/82956/2007, FCT, Portugal

## 611

**A 5-day high fat high calorie diet impairs insulin sensitivity in healthy, young, lean male Dutch Surinamese South Asians but not in Dutch Caucasians**

L.E.H. Bakker<sup>1</sup>, B. Guigas<sup>2</sup>, T.C.M. Streefland<sup>3</sup>, H. Pijl<sup>3</sup>, A.E. Meinders<sup>1</sup>, I.M. Jazet<sup>1</sup>;

<sup>1</sup>Endocrinology/General Internal Medicine, <sup>2</sup>Molecular Cell Biology,

<sup>3</sup>Endocrinology, LUMC, Leiden, Netherlands.

**Background and aims:** People of South Asian descent, such as the Surinamese South Asian population in The Netherlands, develop type 2 diabetes and cardiovascular diseases at a much younger age and lower BMI compared to Caucasians. Moreover, the incidence and seriousness of these diseases is higher in South Asians than in Caucasians. The underlying cause remains unknown but might be related to differences in fatty acid handling leading to impaired skeletal muscle insulin signalling leading to insulin resistance. The aim of this study was to investigate whether whole body insulin sensitivity and substrate oxidation, and skeletal muscle insulin signalling are different between healthy young lean male South Asians and Caucasians at baseline, and whether the response to a 5-day high fat high calorie (HFHC) diet differs between both ethnicities.

**Materials and methods:** 12 male South Asians and 12 male Caucasians (age  $22.2 \pm 0.65$  vs  $22.1 \pm 0.60$  years ( $p = 0.93$ ), BMI  $20.9 \pm 0.58$  vs  $22.3 \pm 0.59$  kg/m<sup>2</sup> ( $p = 0.11$ )) were studied before and after a 5-day-HFHC-diet, consisting of their regular diet, supplemented with 375 ml cream per day (=1275 kcal/day, 94% fat). Endogenous glucose production (EGP, [6,6-<sup>2</sup>H<sub>2</sub>]-glucose), whole body glucose disposal rate (GDR) and substrate oxidation rates (indirect calorimetry) were measured before and after the diet in basal condition and during a hyperinsulinemic euglycemic clamp (insulin infusion rate step 1: 10 mU/m<sup>2</sup>/min, step 2: 40 mU/m<sup>2</sup>/min). In addition, skeletal muscle biopsies were obtained from the vastus lateralis muscle, in basal and hyperinsulinemic euglycemic conditions for determination of insulin signalling.

**Results:** In step 1 no differences were found in our major endpoints. In step 2 EGP decreased from  $11.6 \pm 1.0$  to  $10.2 \pm 0.5$   $\mu\text{mol/kg}_{\text{LBM}}/\text{min}$  ( $p = 0.01$ ) in the South Asian group, but this was at higher serum insulin levels due to a lower metabolic clearance rate of insulin as compared to Caucasians. Despite the higher serum insulin levels, GDR in step 2 decreased from  $53.1 \pm 2.2$  to  $41.8 \pm 2.0$   $\mu\text{mol/kg}_{\text{LBM}}/\text{min}$  ( $p < 0.001$ ) after a 5-day-HFHC-diet. This appeared to be due to a decrease in non-oxidative glucose disposal rate (NOGD) in step 2 ( $35.8 \pm 3.1$  to  $20.6 \pm 2.9$   $\mu\text{mol/kg}_{\text{LBM}}/\text{min}$  ( $p < 0.001$ )). EGP and GDR in the Caucasian group did not change after the diet. No differences were found in oxidative glucose disappearance and lipid oxidation rates. In addition, no major alterations in skeletal muscle insulin signalling pathways were detected, both at baseline and during insulin stimulation.

**Conclusion:** A 5-day-HFHC-diet already impaired insulin stimulated non-oxidative glucose disposal in apparently healthy, young, lean male South Asians, whereas no such effect was observed in matched Caucasians. Since no differences were found in insulin signalling, the decrease in insulin sensitivity in South Asians is possibly due to mitochondrial dysfunction.

Clinical Trial Registration Number: 2473

## 612

**Pancreatic exocrine function and morphology in patients with type 2 diabetes**

I. Miñambres, A. Farré, C. Pérez, M. Cortés, J. Monill, C. Martínez, A. Pérez; Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

**Background and aims:** Diabetes mellitus has been associated with chronic pancreatitis. However, in type 2 diabetes, information regarding morphological and functional changes of the exocrine pancreas remains scarce. Our aim was to study functional and morphological characteristics of the exocrine pancreas in patients with type 2 diabetes.

**Materials and methods:** We included 23 subjects (65% men) with type 2 diabetes. Mean age was  $64 \pm 8.6$  years, mean diabetes duration was  $13 \pm 11$  years, and 65% of the subjects were treated with insulin. Morphological and functional characteristics of the exocrine pancreas were evaluated by means of secretin-magnetic resonance cholangiography and measurement of fecal elastase. Pancreas morphology was classified according to Cambridge classification and pancreatic insufficiency was determined according to the volume of fluid secreted into the duodenum in response to secretin. Fecal elastase values  $> 200$  ug/g were considered normal. Statistical analysis included Chi-squared test and Mann-Whitney test.

**Results:** Fourteen subjects (61%) presented morphological changes suggestive of chronic pancreatitis (6 severe, 5 moderate, 3 mild). Six patients (26%) presented mild pancreatic insufficiency according to the degree of duodenal repletion after stimulation with secretin. Median concentrations of fecal elastase were 451 ug/g (62-500) and only two patients had levels  $< 200$  ug/g. Pharmacological treatment duration was higher in subjects with morphological signs of chronic pancreatitis than in subjects without (14 (2-31) years vs. 6 (0,25-23) years;  $p = 0,043$ ). No associations were found between morphological signs of chronic pancreatitis and sex, age, diabetes duration, type of treatment for diabetes, the presence of pancreatic insufficiency or fecal elastase levels.

**Conclusion:** Most patients with type 2 diabetes present morphological changes of the exocrine pancreas suggestive of chronic pancreatitis. Functional repercussion is less frequent.



## PS 041 Hypoglycaemia: relation to therapy

613

### A 5 year study of severe treatment-induced hypoglycaemia in diabetes: patient characteristics and first year mortality

A.C. Huskinson<sup>1</sup>, M.J. Bottomley<sup>1</sup>, L. Clapham<sup>2</sup>, C. James<sup>2</sup>, S.H. Alzahrani<sup>1</sup>, R. King<sup>1</sup>, R.A. Ajjan<sup>1</sup>;

<sup>1</sup>Division of Diabetes and Cardiovascular Research, Multidisciplinary Cardiovascular Research Centre, Leeds Institution of Genetics, Health and Therapeutics, University of Leeds, UK, <sup>2</sup>Leeds Teaching Hospitals Trust, Leeds, UK.

**Background and aims:** Hypoglycaemia is a common complication of diabetes treatment and can be life-threatening. The aim of this work was to establish the characteristics of individuals with diabetes in the community sustaining severe hypoglycaemia requiring emergency services intervention. **Materials and methods:** This study attempted to record ambulance call outs for severe hypoglycaemia over a period of 5.5 years amongst a population of approximately 28,000 subjects with diabetes.

**Results:** A total of 1312 episodes were recorded in 858 subjects, with 55% occurring in individuals with type 1 diabetes (T1DM), 39% in type 2 diabetes (T2DM) and a minority unclassified. Two third of T1DM subjects were males, whereas gender distribution was equal amongst T2DM individuals. Mean age of T1DM subjects was 48.7 years (range: 8–95), whereas T2DM subjects were older at 72.8 years (range: 32–97). Of the T2DM subjects, 27% were not on insulin treatment with the majority receiving a sulphonylurea (90%). Mean capillary blood glucose ( $\pm$ SEM) at arrival of emergency services was lower in T1DM compared with T2DM subjects ( $1.71 \pm 0.32$  mmol/L and  $1.96 \pm 0.38$  mmol/L, respectively;  $p < 0.001$ ). In T2DM individuals, the severity of hypoglycaemia in insulin users and non-insulin users was similar at  $1.93 \pm 0.50$  mmol/L and  $1.98 \pm 0.55$  mmol/L, respectively ( $p = 0.51$ ). HbA<sub>1c</sub> was higher in T1DM than T2DM subjects ( $66 \pm 1$  mmol/mol and  $60 \pm 1$  mmol/mol, respectively;  $p < 0.001$ ), and insulin users in T2DM had significantly higher HbA<sub>1c</sub> compared with those not on this therapy. Around one third of patients self-reported hypoglycemic unawareness. Basal-bolus regime was more commonly used in T1DM subjects compared with T2DM (61.5% and 13.7%, respectively;  $p < 0.001$ ). In contrast, a mixed insulin regime was more frequently used in T2DM compared with T1DM individuals (68.6% and 24.4%, respectively;  $p < 0.001$ ). Mortality within one year of the severe hypoglycemic event was 17.1% in the whole population, which is comparable to the one year mortality of 15.6% following myocardial infarction in diabetes subjects in our area.

**Conclusion:** This observational study demonstrates that severe hypoglycaemia is a common complication in both T1DM and T2DM, and frequently occurs in non-insulin treated individuals, the majority of which are on sulphonylurea therapy. We show high mortality in the first year following severe hypoglycaemia, although it is unclear at present whether low blood glucose directly contributes to death in these individuals. Future work is warranted to establish whether specialist intervention following severe hypoglycaemia reduces further events or mortality in this high risk population.

614

### Substantial reduction in the frequency of severe hypoglycaemic events in children and adolescents with type 1 diabetes in Denmark in the period 2008–2011

S. Fredheim<sup>1</sup>, A. Johansen<sup>2</sup>, L.B. Nielsen<sup>1</sup>, M.-L.M. Andersen<sup>1</sup>, J. Johannesen<sup>1</sup>, H.B. Mortensen<sup>1</sup>, J. Svensson<sup>1</sup>;

<sup>1</sup>Pediatric Ward, Herlev University Hospital, <sup>2</sup>Pediatric Ward, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark.

**Background and aim:** Recent advances in diabetes treatment regimens call for an evaluation of current practice. This national study aimed at describing the metabolic outcome and the frequency of acute adverse outcome in children and adolescents with Type 1 Diabetes (T1D) according to treatment modalities within the recent 4 years in Denmark.

**Materials and methods:** A national register-based study including children and adolescents < 17 years from Denmark diagnosed with T1D followed from 2008 till 2011 both year included. Clinical data was prospectively recorded, including events of severe hypoglycaemia (SH) and annual glycated

haemoglobin A1c (HbA1c). SH was defined as unconsciousness and/or seizures and a blood glucose level of < 3.5 mmol/L. A negative binominal model was used to evaluate the current treatment modality to the frequency of SH over the following 4 years.

**Results:** 1271 children (girls 49.2 %, boys 50.8 %) were included in the study. Data completeness for individual and annual HbA1c recordings in the period has increased from 2008 (80 %) till 2012 (86 %), as did recordings of SH in 2008 (81 %) till 2012 (87 %). We observe a decreasing frequency of SH with -0.38 % (SEM 0.05,  $p < 0.001$ ) pr. year in our population. Stratified according to the time period, we observe a decreasing frequency of SH: 9.7 % (2008), 8.4 % (2009), 5.5 % (2010) and 4.6 % (2011). The overall mean HbA1c over the respective 4 years do not significantly improve (8.31 % 2008, 8.26 % 2009, 8.32 % 2010, 8.35 % 2011). There is a significant increasing frequency of SH associated to increasing HbA1c levels ( $p = 0.03$ ), however when adjusting for treatment modality this finding is no longer present. In the same period, a decreasing number of patients are on multiple daily insulin injections: 78.1 % (2008) to 51.6 % (2011), and an increasing number of patients are treated with continuous subcutaneous insulin injections (CSII): 21.9 % (2008) to 48.4% (2011). We observe a significant decreasing frequency of SH for patients on CSII compared to MDI, -0.032 (SEM=0.13,  $p = 0.014$ ).

**Conclusion:** Although long-term HbA1c-levels are unaltered, we observe an overall substantial decrease in severe hypoglycaemic events in Denmark. Despite an increasing number of patients on CSII treatment, these patients experience fewer SH events, also as HbA1c improves.

615

### Prospectively planned meta-analysis comparing hypoglycaemia rates of insulin degludec with those of insulin glargine in all patients and an elderly ( $\geq 65$ year) subgroup

S.C.L. Gough<sup>1</sup>, R. Ratner<sup>2</sup>, C. Mathieu<sup>3</sup>, S. Del Prato<sup>4</sup>, B. Bode<sup>5</sup>, H. Mersebach<sup>6</sup>, L. Endahl<sup>6</sup>, B. Zinman<sup>7</sup>;

<sup>1</sup>Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK, <sup>2</sup>MedStar Health Research Institute, Hyattsville, USA, <sup>3</sup>Katholieke Universitet Leuven, Belgium, <sup>4</sup>University of Pisa, Italy, <sup>5</sup>Atlanta Diabetes Associates, Atlanta, USA, <sup>6</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>7</sup>Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada.

**Background and aims:** Insulin degludec (IDeg), a new basal insulin that forms soluble multi-hexamers after sc injection, has an ultra-long and stable glucose-lowering effect with low day-to-day variability. This pre-specified analysis compared the hypoglycaemia rate with IDeg vs insulin glargine (IGlar) in patients with type 1 and type 2 diabetes (T1D and T2D).

**Materials and methods:** Hypoglycaemia was defined as self-reported confirmed hypoglycaemia (plasma glucose < 3.1 mmol/l or severe hypoglycaemia requiring assistance) and nocturnal confirmed hypoglycaemia (00:01–05:59, inclusive). Rates were analysed with a negative binomial regression model on patient-level data. All open-label, randomised, treat-to-target phase 3a trials of 26 or 52 weeks in which once-daily IDeg ( $n = 2899$ ) was compared to IGlar ( $n = 1431$ ) in T1D (2 trials) and T2D (5 trials) were included. We also present a pre-planned sub-analysis in subjects aged  $\geq 65$  years (IDeg  $n = 632$ ; IGlar  $n = 283$ ).

**Results:** IDeg was associated with statistically significant lower rates of overall and nocturnal hypoglycaemia vs IGlar in T2D, and of nocturnal hypoglycaemia in T1D (Table). A significant advantage of IDeg was also evident for the pooled population (T1D+T2D). Analyses of the maintenance period (stable glycaemia and insulin dose [obtained from 16 weeks onward]), demonstrated a more pronounced advantage of IDeg. Additional analysis revealed significantly less severe hypoglycaemia with IDeg vs IGlar in insulin-naïve T2D (rate ratio [RR] 0.14 [95% CI: 0.03; 0.70]). Across trials, 21.1% of all subjects (6.4% T1D, 25.3% T2D) were  $\geq 65$  years. Consistent with confirmatory analyses in the overall population, elderly subjects had fewer confirmed hypoglycaemic episodes with IDeg vs IGlar. Confirmed hypoglycaemia was numerically lower in IDeg-treated elderly subjects (RR IDeg/IGlar: 0.82 [95% CI: 0.66; 1.00]). Nocturnal hypoglycaemia rates were significantly lower with IDeg (RR 0.65 [95% CI: 0.46; 0.93]).

**Conclusion:** Treatment with IDeg provides important clinical advantages with significantly lower rates of overall and nocturnal hypoglycaemia vs IGlar at similar HbA<sub>1c</sub> levels. Elderly subjects treated with IDeg experience a numerically lower rate of confirmed hypoglycaemia and a significantly (35%) lower nocturnal hypoglycaemia rate with IDeg vs IGlar, confirming that of individual trial data from the IDeg clinical program. Lower rates of hypoglycaemia associated with IDeg may be of particular benefit in the elderly population on insulin.

## Meta-analysis of confirmed hypoglycaemia for IDeg vs IGLar in all subjects

	Hypoglycaemia rate ratio (IDeg/IGlar)		Nocturnal hypoglycaemia rate ratio (IDeg/IGlar)	
	Total period	Maintenance period <sup>a</sup>	Total period	Maintenance period <sup>a</sup>
T2D	0.83 [0.74; 0.94]*	0.75 [0.66; 0.87]*	0.68 [0.57; 0.82]*	0.62 [0.49; 0.78]*
T2D: insulin-naïve	0.83 [0.70; 0.98]*	0.72 [0.58; 0.88]*	0.64 [0.48; 0.86]*	0.51 [0.36; 0.72]*
T1D	1.10 [0.96; 1.26]	1.02 [0.88; 1.19]	0.83 [0.69; 1.00]	0.75 [0.60; 0.94]*
T1D+T2D <sup>b</sup>	0.91 [0.83; 0.99]*	0.84 [0.75; 0.93]*	0.74 [0.65; 0.85]*	0.68 [0.58; 0.80]*

<sup>a</sup>From 16 weeks onwards; <sup>b</sup>The pooled analysis was the primary endpoint; [ ] = 95% confidence intervals; \**p*<0.05

Supported by: Novo Nordisk

## 616

### The risk of first severe hypoglycaemia depends on the type of basal insulin but does not differ between patients with type 1 and type 2 diabetes: The EpiHypo Study

P. Korhonen<sup>1</sup>, J. Haukka<sup>1</sup>, F. Hoti<sup>1</sup>, P. Erästä<sup>1</sup>, S. Mäkimattila<sup>2</sup>, T. Saukkonen<sup>2</sup>; <sup>1</sup>EPID Research, Espoo, Finland, <sup>2</sup>Novo Nordisk, Espoo, Finland.

**Background and aims:** While incidence of severe hypoglycaemia (SH) is well documented in type 1 diabetes (T1D), there is limited information on frequency of SH in type 2 diabetes patients (T2D) receiving insulin therapy. The aim of this retrospective follow-up study was to investigate the incidence and risk factors of hospitalisation and secondary health care visits due to SH in patients with T1D or T2D using NPH, glargine (IGla), or detemir (IDet) insulin.

**Materials and methods:** Data was obtained by linking Finnish nationwide health care databases. Study population included all diabetes patients (n=137 994) entitled to special reimbursement for diabetes medication and who had purchased insulin during 2000–2009. The present analysis comprised those patients (n=75 682) who had not used insulin before year 2000. Stratified incidence rates with 95% CIs were calculated. Hazard ratios (HR) were estimated by Cox's proportional hazards model, adjusted for age, gender, prior and current use of other insulins and sulfonylureas, switch between insulins, history of hypoglycaemia, years from diagnosis, calendar year of the start of follow-up and hospital district.

**Results:** We identified altogether 842 and 2732 first incident SHs in patients with T1D and T2D, respectively. Incidence of first SH was 1.74 (1.64–1.86) in T1D and 1.91 (1.85–1.98) in T2D per 100 patient years (ptys). Type of diabetes was not significantly associated with risk of first SH (HR 1.11, CI 0.99–1.25, *p*=0.079 for T2D vs T1D), however the risk of recurrence of SH was significantly higher in T2D (1.40, CI 1.24–1.58, *p*<0.001) compared to T1D. The results for the incidence and risk of first SH during the use of NPH, IGla or IDet are shown in Table.

	Ptyrs	Events	Incidence (95% CI) / 100 ptyrs	HR (95% CI) vs. NPH	p value	HR (95% CI) vs. glargine	p value
T1D							
NPH	19563	454	2.32 (2.12 - 2.54)	1			
IGla	18850	272	1.44 (1.28 - 1.63)	0.73 (0.60 - 0.89)	0.002	1	
IDet	9520	116	1.22 (1.02 - 1.46)	0.67 (0.52 - 0.88)	0.003	0.92 (0.74 - 1.16)	0.490
T2D							
NPH	96164	1937	2.01 (1.93 - 2.11)	1			
IGla	33958	660	1.94 (1.80 - 2.10)	0.96 (0.85 - 1.08)	0.460	1	
IDet	10120	135	1.33 (1.13 - 1.58)	0.74 (0.60 - 0.90)	0.003	0.77 (0.64 - 0.93)	0.006

**Conclusion:** Based on the EpiHypo register study, risk of hospitalisation due to SH is similar or even increased in insulin-treated T2D compared to T1D. In T1D, both long-acting insulin analogues were associated with lower risk of SH compared to NPH. In T2D, risk of hospitalisation due to SH was significantly lower during use of IDet compared to both IGla and NPH.

Clinical Trial Registration Number: U1111-1120-7164

Supported by: Novo Nordisk

## 617

### Lower rates of overall and nocturnal hypoglycaemia with insulin degludec vs insulin glargine during treatment intensification in type 2 diabetes patients: a meta-analysis

A.J. Garber<sup>1</sup>, S. Colagiuri<sup>2</sup>, H.W. Rodbard<sup>3</sup>, S. Rasmussen<sup>4</sup>, N. Lassota<sup>4</sup>, S.C.L. Gough<sup>5</sup>;

<sup>1</sup>Department of Medicine, Baylor College of Medicine, Houston, USA,

<sup>2</sup>University of Sydney, Australia, <sup>3</sup>Endocrine and Metabolic Consultants,

Rockville, USA, <sup>4</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>5</sup>University of

Oxford, UK.

**Background and aims:** Current basal insulin treatments increase the risk of hypoglycaemia when HbA<sub>1c</sub> levels approach normoglycaemia. Insulin degludec (IDeg) is a new basal insulin that forms soluble multi-hexamers upon subcutaneous injection, resulting in an ultra-long action profile with a low day-to-day variability.

**Materials and methods:** We analysed whether these characteristics of IDeg would improve glycaemic control and result in lower rates of hypoglycaemia compared to insulin glargine (IGlar) in patients with type 2 diabetes (T2D) in moderately good control at baseline (HbA<sub>1c</sub> of 7.5–8.5%). Changes in HbA<sub>1c</sub> and fasting plasma glucose (FPG) were analysed with linear models, and rates of hypoglycaemia with a negative binomial regression model. Hypoglycaemia was defined as self-reported confirmed hypoglycaemia (PG <3.1 mmol/L) or severe episodes if requiring assistance; nocturnal confirmed hypoglycaemia was defined as onset between 00:01 and 05:59 inclusive. Analysis included patient-level data from all five open-label randomised treat-to-target phase 3a trials in T2D (26 or 52 weeks) in which IDeg (n=930) or IGlar (n=446) were given once daily.

**Results:** HbA<sub>1c</sub> decreased from 8.0% at baseline in both groups to 7.0 versus 6.9% at end of trial for IDeg versus IGlar, respectively (treatment difference: 0.08%-points [95% CI: -0.01; 0.18]; *p*=0.08). Observed FPG decreased from 9.0 to 6.1 mmol/L for IDeg and from 8.9 to 6.3 mmol/L for IGlar, achieving a statistically significant estimated treatment difference of -0.36 mmol/L [95% CI: -0.60; -0.11]; *p*<0.01. The rate ratio (IDeg:IGlar) of confirmed hypoglycaemia (0.80 [95% CI: 0.67; 0.96]; *p*=0.02) and nocturnal hypoglycaemia (0.69 [95% CI: 0.51; 0.92]; *p*=0.01) were both statistically significantly lower for IDeg versus IGlar.

**Conclusion:** Patients with T2D with baseline HbA<sub>1c</sub> of 7.5–8.5% treated with IDeg showed comparable improvement in HbA<sub>1c</sub>, significantly greater reduction in FPG, and significant reduction (20%, 31%) in rates of overall and nocturnal hypoglycaemia, respectively, compared to IGlar.

Supported by: Novo Nordisk

## 618

### Hypoglycaemia risk in type 2 diabetes: a multivariate analysis comparing exenatide once weekly with insulin glargine experience in a large clinical trial dataset

S.K. Paul<sup>1</sup>, K. Klein<sup>1</sup>, D. Maggs<sup>2</sup>, J.H. Best<sup>2</sup>;

<sup>1</sup>University of Queensland, Brisbane, Australia, <sup>2</sup>Medical Research and Development, Amylin Pharmaceuticals, San Diego, USA.

**Background and aims:** The time-varying effects of risk factors on hypoglycaemia in patients with type 2 diabetes are not known. This post-hoc analysis explored the possible association of anthropometric and clinical risk factors with hypoglycaemia risk in patients treated with either exenatide once weekly (EQW) or insulin glargine (IG).

**Materials and methods:** Data over 52 wks from 3 controlled trials (DURATION-1, -2, -3) involving 541 EQW patients (54% male, mean±SD baseline age 55±10 y, weight [WT] 93±20 kg, HbA<sub>1c</sub> 8.4±1.1%, diabetes duration 6 y) and 223 IG patients (55% male, mean±SD baseline age 58±9 y, WT 91±16 kg, HbA<sub>1c</sub> 8.3±1.0%, diabetes duration 6 y), were analyzed. Multivariate mixed-effects models were fitted to capture time-varying effects of risk factors on hypoglycaemia incidence (including major, minor and symptomatic), adjusting for age, gender, diabetes duration, concomitant metformin plus sulphonylurea (MET+SU), and longitudinal measures of HbA<sub>1c</sub>, WT, blood pressure, and lipids.

**Results:** MET+SU was used by 26% EQW and 31% IG patients. Hypoglycaemia occurred more often with IG (51% IG and 19% EQW patients experienced ≥ 1 hypoglycaemia episode) and with greater frequency with SU background (SU: 37% EQW and 66% IG; non-SU: 12% EQW and 45% IG). Multivariate analysis showed SU use increased hypoglycaemia risk 3–4 fold for IG and EQW patients. Lower HbA<sub>1c</sub> was more closely associated with

increased incidence and frequency of hypoglycaemia in IG-treated patients than in EQW-treated patients (hypoglycaemia risk with HbA1c  $\leq 6.5$  %: IG, 30%; EQW, 3%). WT change did not influence hypoglycaemia risk with IG patients but EQW-induced WT loss reduced risk (2.5 kg reduction reduced risk 6%).

**Conclusion:** This analysis provides estimates of the effects of time-varying glycaemic control and weight changes on the risk of hypoglycaemia observed with basal insulin and EQW, a long-acting GLP-1 receptor agonist. The analysis confirms the higher risk of hypoglycaemia observed with IG treatment, particularly with more intensified glycaemic control, and that background SU use is a significant driver of hypoglycaemia with both treatments.

*Clinical Trial Registration Number:* NCT00637273

*Supported by:* Amylin Pharmaceuticals, Inc.

## 619

### The impact of insulin type on severe hypoglycaemia events requiring inpatient and emergency department care in patients with type 2 diabetes

M. Solomon<sup>1</sup>, S. Vijan<sup>2</sup>, F. Forma<sup>3</sup>, R. Conrad<sup>4</sup>, N. Summers<sup>4</sup>, D. Lakdawalla<sup>5</sup>;

<sup>1</sup>Stanford University School of Medicine, Stanford, <sup>2</sup>University of Michigan School of Medicine, Ann Arbor, <sup>3</sup>sanofi-aventis US, Bridgewater, <sup>4</sup>Precision Health Economics, Los Angeles, <sup>5</sup>University of Southern California, Los Angeles, USA.

**Background and aims:** Although clinical trials suggest that basal analogues have a lower risk of hypoglycemia than other forms of insulin, conclusive data are lacking. We evaluated the risk from different insulin types on severe hypoglycemia (SHG) events requiring inpatient (IP) or emergency department (ED) care in patients with Type 2 diabetes (DM).

**Materials and methods:** Using a large database of privately-insured working-age US adults from 1998–2009, we conducted a retrospective cohort study of DM patients newly started on insulin after at least 6 months of treatment with oral anti-diabetic medications (OADs). We measured SHG events after insulin initiation occurring in the IP or ED setting. Patients were classified into an insulin group based on: (1) the most frequently used insulin type prior to the SHG event for those with SHG events; or (2) the most frequently used insulin overall during our observation period for patients without SHG events. Multivariate Cox proportional hazard regression models assessed the association between insulin type and the risk of subsequent SHG events, controlling for demographics, comorbidities, and the number and type of concomitantly prescribed OADs. Sensitivity analyses of more inclusive definitions of SHG events were conducted.

**Results:** The cohort included 8,626 patients (age 53.5 years; 55% female) with DM followed for an average of 4.0 years after insulin initiation. Of these, 161 (1.9%) had a SHG event at an average of 3.1 years after insulin initiation. Patients with SHG events were slightly older (56.4 years vs. 53.4 years), used a similar number of OADs (1.1 vs. 1.2), and were more likely to have multiple comorbidities compared to those without SHG events. Overall, the most frequently used insulin type was glargine (55%), followed by rapid-acting insulin (23%), isophane insulin (NPH) (9.2%), detemir (6.9%) and premixed insulin (6.6%). In multivariate Cox models, premixed insulin (HR 2.07;  $p < 0.01$ ), isophane insulin (NPH) (HR 2.01;  $p < 0.01$ ), and rapid acting insulin (HR 2.78;  $p < 0.01$ ) had a higher risk of SHG events compared to glargine, and detemir showed a non-significant trend toward more SHG events (HR 1.20;  $p = 0.73$ ). These results were unchanged in sensitivity analyses of more inclusive definitions of SHG events.

**Conclusion:** Among patients with DM who initiate insulin therapy, we found glargine users had lower rates of SHG events requiring IP or ED care compared to users of other insulin formulations. This analysis suggests that differences in the risk of SHG events may exist for various insulin types. Future research should examine the impact and burden of hypoglycemia events of different severity levels.

*Supported by:* Sanofi-Aventis US

## 620

### Accuracy of nocturnal continuous glucose monitoring (CGM) in type 1 diabetes patients at high risk of severe hypoglycaemia

C. Bay<sup>1</sup>, P. Lommer Kristensen<sup>1</sup>, U. Pedersen-Bjergaard<sup>1</sup>, L. Tarnow<sup>2</sup>, B. Thorsteinsson<sup>1,3</sup>;

<sup>1</sup>Endocrinology Section, Dept. of Cardiology, Nephrology and Endocrinology, Hillerød Hospital, Hillerød, <sup>2</sup>Steno Diabetes Center, Gentofte, <sup>3</sup>Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

**Background and aims:** A reliable method to detect biochemical nocturnal hypoglycaemia is needed, especially for patients with recurrent severe hypoglycaemia. Continuous Glucose Monitoring (CGM) may reveal these episodes. This study aimed to evaluate reliability of nocturnal CGM in patients with type 1 diabetes at high risk of severe hypoglycaemia.

**Materials and methods:** We here report CGM data originating from a two-year randomised, open-labelled, crossover multicentre trial designed to investigate the ability of insulin detemir / insulin aspart vs human NPH insulin / soluble insulin to reduce the incidence of severe hypoglycaemia in high-risk patients. Inclusion criteria were age  $\geq 18$  years, type 1 diabetes  $\geq 5$  years,  $\geq 2$  episodes of severe hypoglycaemia during the preceding year, and basal-bolus treatment with human insulin or insulin analogues. The patients underwent CGM (Medtronic MiniMed Guardian® REAL-Time CGMS using Sof-sensor®) for 2 x 2 nights at the diabetes clinic. CGM was calibrated with finger-stick blood glucose measurements before bedtime and blinded to patients and study personnel. Blood was drawn hourly from an i.v. catheter from 23:00 - 7:00 for plasma glucose (PG) measurements, which was considered gold standard. Nocturnal hypoglycaemia was defined by three thresholds:  $<4$ ,  $<3$  and  $<2.2$  mmol/L. A valid episode of hypoglycaemia measured by CGM lasted  $\geq 20$  min.

**Results:** A total of 72 of 110 patients accepted to stay overnight (46 males, age  $54 \pm 12$  years (mean  $\pm$  SD), HbA1c  $8.1 \pm 1.1$ , duration of diabetes  $30 \pm 14$  years), totally 217 nights yielding 1786 paired PG and CGM measurements. Fifty-three per cent of the patients experienced at least one hypoglycaemic night with PG  $< 4$  mmol/L. Prevalences of nocturnal hypoglycaemia according to the three thresholds  $<4$ ,  $<3$  and  $<2.2$  mmol/L were 28%, 16%, 3% for PG and 33%, 12%, 3% for CGM. The sensitivity of CGM according to the three thresholds was 65% (95% CI: 53–77), 40% (24–56) and 17% (0–46), respectively. If only hypoglycaemic nights were taken into account, CGM missed detection of 35% of hypoglycaemia  $<4$ , 60%  $<3$  and 83%  $<2.2$  mmol/L. The correlation coefficient between plasma glucose and CGM recordings was 0.82 (Spearman's rho;  $p < 0.001$ ). Bland-Altman analysis demonstrated that CGM on average underestimated PG by 1.1 mmol/L (0.9–1.2;  $p < 0.001$ ). Of all the paired values 94% were within 95% CI limits, however 2 SD were 5 mmol/L. The mean absolute relative difference (MARD) (%) was higher in the hypoglycaemic range ( $\leq 3.9$  mmol/L) compared to the normo- and hyperglycaemic ranges (4–9.9 mmol/L and  $\geq 10$  mmol/L) as determined by one-way ANOVA ( $p < 0.001$ , Bonferroni correction): 45 (37–53), 23 (22–25) and 20 (19–21), respectively.

**Conclusion:** Reliability of CGM using Sof-sensor® for detecting nocturnal hypoglycaemia in type 1 diabetic patients at high risk of severe hypoglycaemia is inadequate. However, CGM may benefit some high-risk patients since it provides comprehensive night-time glucose profiles and since nocturnal hypoglycaemia is usually unrecognized.

*Clinical Trial Registration Number:* NCT00346996

*Supported by:* Research Foundation of Hillerød Hospital, Denmark

## 621

### Characteristics and costs of individuals experiencing severe hypoglycaemia requiring emergency ambulance assistance in the community

K. Khunti<sup>1</sup>, H. Fisher<sup>2</sup>, S. Paul<sup>3</sup>, I. Mohammad<sup>4</sup>, M.J. Davies<sup>5</sup>, A.N. Siriwardena<sup>6</sup>;

<sup>1</sup>Department of Health Sciences, University of Leicester, UK, <sup>2</sup>School of Social and Community Medicine, University of Bristol, UK, <sup>3</sup>Queensland Clinical Trials and Biostatistics Centre, University of Queensland, Brisbane, Australia, <sup>4</sup>East Midlands Ambulance Service NHS Trust, Nottingham, UK, <sup>5</sup>Department of Cardiovascular Sciences, University of Leicester, UK, <sup>6</sup>School of Health and Social Care, University of Lincoln, UK.

**Background and aims:** Hypoglycaemia causes considerable a burden to individuals the healthcare providers. The aim of this study was to examine clinical



cal characteristics of individuals requiring emergency medical assistance by ambulance services for an episode of severe hypoglycaemia and to estimate provider costs of hypoglycaemia.

**Materials and methods:** Routinely collected information was retrieved for all episodes of severe hypoglycaemia attended to by the emergency ambulance services for a population of 367,051 people, including 75,603 people with diabetes, in Nottinghamshire and Derbyshire, UK, between 01/11/10 to 28/02/11. A total of 90,435 emergency calls were received in the study period, of which 523 (0.6%) were recorded as severe hypoglycaemia. The time to response, on-site treatment and hospitalisation were recorded along with standard clinical and blood glucose (BG) measures. Ambulance services costs were calculated.

**Results:** The mean (SD) [proportion  $\leq$  3.2 mmol/L] pre and post-treatment BG levels were 1.9 (0.9) mmol/L [92%] and 6.5 (3.1) mmol/L [3%] respectively, 74% were under insulin treatment, 28% had nocturnal hypoglycaemia, and 153 (32%) individuals were transported to hospital. Lower pre-treatment BG ( $p < 0.01$ ) and Glasgow Coma Scale scores ( $p = 0.05$ ) were observed in insulin treated individuals in comparison to non-insulin treated individuals. No significant differences in individual characteristics were observed for other clinical measurements: post-treatment blood glucose ( $p = 0.39$ ), systolic blood pressure ( $p = 0.28$ ), diastolic blood pressure ( $p = 0.64$ ) and heart rate ( $p = 0.93$ ). Non insulin treatment was an independent predictor of transportation to hospital ( $p < 0.01$ ). Median time from allocation of call to departure of scene by ambulance services was 39 and 59 minutes for those transported and not transported to hospital respectively, translating to costs of £92 and £139 respectively. The median time from allocation to handing over patients to emergency staff was 75 minutes, equating to a cost of £176.

**Conclusion:** The majority of cases of severe hypoglycaemia are successfully treated at the scene by the emergency ambulance services. Insulin treated and non insulin treated individuals do not differ by clinical characteristics, however non insulin treated individuals were more likely to be transported to hospital. Further studies are needed into the effect of prehospital ambulance care by treatment type on subsequent outcomes.

*Supported by: Novo Nordisk*

## PS 042 Hypoglycaemia: mechanisms and effects

622

### Recurrent hypoglycaemia increases microRNA expression in the anterior cingulate cortex of brain

W.J. Marsh<sup>1</sup>, E. Ogunnowo-Bada<sup>2</sup>, C.H. Riches<sup>2</sup>, G. Yeo<sup>1</sup>, M.L. Evans<sup>2</sup>;

<sup>1</sup>Department of Clinical Biochemistry, <sup>2</sup>Department of Medicine, Institute of Metabolic Science, Cambridge, UK.

**Background and aims:** Some patients with type 1 diabetes (T1D) lose counterregulatory responses (CRR) to low blood glucose, associated with hypoglycaemia unawareness and increased risk of severe hypoglycaemia. Although the mechanisms remain unclear, hypoglycaemia itself can lead to down-regulation of CRR- a type of "stress desensitisation". We have recently implicated the anterior cingulate cortex (ACC), part of the brain's limbic system which regulates stress responses, in this process. Specifically, hypoglycaemia-induced activation of ACC is lost following recurrent hypoglycaemia (RH). We hypothesised that recurrent episodes of hypoglycaemia would result in discrete changes in gene expression within the ACC, explaining this loss of activation.

**Materials and methods:** 2 groups of chronically-catheterised (carotid and jugular) Sprague-Dawley rats underwent 3 sequential days of s.c. insulin (10/8/6 U/kg Humulin S) with regular sampling of plasma glucose and dextrose infusion as needed. One group was allowed to fall into hypoglycaemic range ( $2.7 \pm 0.1$  mM;  $n = 5$ ) on each of the 3 study days. A control group was maintained at euglycaemia ( $7.4 \pm 0.2$  mM;  $n = 5$ ). Of note, this study design ensures that exposure to exogenous insulin was matched between both groups. The morning following the last insulin injection, brains were harvested, the ACC micro-punched from frozen brain slices, and RNA collected for hybridisation on Affymetrix Rat Gene ST 1.0 microarrays. CEL files were analysed on Genespring 11. Genes were considered significantly altered at  $P > 0.05$  and fold change  $> 1.1$ .

**Results:** In the ACC, 366 genes were up-regulated and 420 genes down-regulated after RH, mostly with relatively subtle alterations in keeping with a physiologically-relevant (mal)adaptation. Of note, 6 of the 9 genes showing the greatest increase with RH were micro-RNAs, a class of regulatory RNA involved in silencing gene expression. In total, 17 up-regulated and 5 down-regulated species encoded micro-RNAs.

**Conclusion:** In summary, RH led to subtle changes in gene expression including increases in micro-RNA in the ACC, a stress regulatory area. Our data suggest that loss of CRR defences against hypoglycaemia in diabetes might be a result of novel epigenetic changes within the brain's stress circuitry.

ID	Name	Fold Change
mir-379	microRNA 379	1.60
mir-30	microRNA 30c-1	1.60
mir-329	microRNA 329-1	1.46
mir-24	microRNA 24-1	1.37
mir-544	microRNA 544	1.35
mir-9	microRNA 9-1	1.30
mir-29	microRNA 29b-2	1.29
mir-134	microRNA 134	1.28
mir-374	microRNA 374a	1.23
mir-221	microRNA 221	1.20
mir-95	microRNA 95	1.20
mir-433	microRNA 433	1.18
mir-21	microRNA 21	1.18
mir-668	microRNA 668	1.14
mir-27	microRNA 27a	1.12
let-7	microRNA let-7a-1	1.10
mir-154	microRNA 1185-2	1.10
mir-17	microRNA 17	-1.11
mir-760	microRNA 760	-1.12
mir-132	microRNA 132	-1.12
mir-328	microRNA 328	-1.13
mir-504	microRNA 504	-1.16

**Table 1: list of all miRNAs differentially regulated in the ACC upon 3 days of recurrent hypoglycaemia**

Supported by: Wellcome Trust, MRC

## 623

**Effect of hypoglycaemia on QT interval, parameters of heart rate variability and frequency of arrhythmias in children and adolescents with type 1 diabetes mellitus**

D. Laptev, T. Kuraeva, V. Peterkova;

Endocrinology Research Center, Moscow, Russian Federation.

**Background and aims:** Hypoglycemia can be associated with the development of life-threatening arrhythmias. The main aim of this study was to determine effect of hypoglycemia on QT interval, heart rate variability (HRV) parameters and frequency of arrhythmias in children and adolescents with type 1 diabetes mellitus (T1DM).

**Materials and methods:** The study included 107 young patients (aged 6–18 years) with T1DM. All participants underwent simultaneous continuous ECG (Holter) and glucose (CGM) monitoring for 24-hours. Automatic calculation of QTc and HRV parameters (SDNN, RMSSD) were performed. All data were averaged for 5 minutes synchronously with CGM. Patients with episodes of hypoglycemia (blood glucose less than 3.5 mmol/l) during the day (7:00–23:00) and night (23:00–7:00) were defined. In these patients length of QTc and RR intervals, parameters of HRV and arrhythmias during hypo- and normoglycemia (BG 5–15 mmol/l) were compared.

**Results:** There were 24 episodes of nocturnal hypoglycemia in 17 participants (15.6%) and 45 episodes of diurnal hypoglycemia in 34 participants (31.8 %). During episodes of nocturnal hypoglycemia there were lengthening of QTc interval (439 vs. 424 ms;  $P<0.05$ ) and reduction in HRV parameters (SDNN

68 vs. 90 ms and RMSSD 48 vs. 79 ms;  $P<0.05$ ) w/o significant increase in heart rate (69 vs. 68 beats/min;  $P=0.29$ ). Same except for the HR was during the day (hypoglycemia vs. normoglycemia respectively: QTc interval 435 vs. 422 ms; SDNN 55 vs. 73 ms; RMSSD 26 vs. 45 ms; heart rate 94 vs. 90 beats/min;  $p<0.05$ ). There were no differences in occurrence of arrhythmia during the night. However daytime frequency of ventricular ectopic events was higher during episodes of hypoglycemia.

**Conclusion:** During episodes of hypoglycemia observed prolongation of the QTc interval, reduction in HRV and there may be increased frequency of arrhythmias in daytime.

## 624

**Gastric bypass surgery is associated with a high risk of hypoglycaemia in morbid obesity**

J.M. Brix<sup>1</sup>, H.P. Kopp<sup>1</sup>, M. Schermann<sup>2</sup>, G.H. Scherthaner<sup>3</sup>, G. Scherthaner<sup>1</sup>;

<sup>1</sup>Department of Medicine I, Rudolfstiftung Hospital, <sup>2</sup>Department of Surgery, Rudolfstiftung Hospital, <sup>3</sup>Department of Medicine II, Medical University of Vienna, Austria.

**Background and aims:** Gastric Bypass (GB) surgery is believed to have the best long term results after bariatric surgery. However, detailed investigations about the risk of hypoglycaemia following various surgical interventions are not available. Thus, we performed a prospective study in a large cohort of patients with morbid obesity (MO).

**Materials and methods:** In total 1127 patients with MO (mean BMI: 43.7±9.4 kg/m<sup>2</sup>; mean age: 38 ±11 years; 80.6% females) were studied. In a longitudinal study 175 patients were evaluated before and 2 years after GB. All patients underwent an oral glucose tolerance test (OGTT; 75g glucose) with measurements of blood glucose (BG) and insulin levels. Hypoglycemia was defined as a BG level ≤50 mg /dl. HOMA-insulin resistance was calculated.

**Results:** Before surgery only 8 out of the 1127 (0.007%) patients showed a hypoglycemic event during the OGTT. In patients included in the longitudinal study hypoglycemia was also rather low (0.02%) before surgery, but post-surgery 72 (22.9%) out of the 314 patients showed a post-challenge BG level ≤50 mg /dl (BG range 20–50mg/dl). In the group of patients who underwent GB surgery (n=175), hypoglycemia was present in 57 patients (32.6%); in the Sleeve Gastrectomy group (n=72), 14 patients (19.4%) were suffering hypoglycaemia but only one patient (2.3%) presented with hypoglycemia in the Gastric Bypass group (n=44). Interestingly none of the patients who had vertical banded gastroplasty (n=23) had hypoglycemia in the oGTT. Patients presenting with hypoglycemia lost more weight (46±16kg vs 40±18kg;  $p=0.016$ ) and had a greater loss in BMI (16.0±5.6kg/m<sup>2</sup> vs 14.1±6.2kg/m<sup>2</sup>;  $p=0.038$ ) than patients without hypoglycemia. After surgery, regarding the oGTT, differences in patients with or without hypoglycaemia are depicted in table1.

**Conclusion:** This is the first prospective study indicating a much higher prevalence of severe hypoglycemia (22.9%) after bariatric surgery, especially after Gastric Bypass surgery. The risk for hypoglycemia is particularly high in those patients with a greater weight loss associated with a low insulin-resistance state but still presenting with high post-challenge insulin levels. In conclusion, a systematic evaluation of glucose and insulin levels after an OGTT 2 years post-surgery may help to identify patients at increased risk for severe hypoglycemia.

Table 1

	Hypoglycemia	No Hypoglycemia	p-value
Glucose fasting, mg/dl	75±7	83±10	$p<0.001$
Glucose 1-hour postprandial, mg/dl	147±48	139±48	$p=0.303$
Glucose 2-hour postprandial, mg/dl	40±7	83±28	$p<0.001$
Insulin fasting, µU/ml	6.7±3.0	10.5±7.1	$p<0.001$
Insulin 1-hour postprandial, µU/ml	162.8±109.3	95.0±73.9	$p<0.001$
Insulin 2-hour postprandial, µU/ml	10.2±7.3	27.7±31.8	$p<0.001$
HbA1c, %	5.3±0.4	5.3±0.3	$p=0.240$
HOMA-InsulinResistance	1.2±0.6	2.2±1.6	$p<0.001$

## 625

**Mild familial hyperinsulinaemic hypoglycaemia due to duplication of the SLC16A1 gene**H.U. Irgens<sup>1,2</sup>, T.T.V. Hoang<sup>1,3</sup>, M.S. Strøm<sup>1,2</sup>, A. Molven<sup>4,5</sup>, S. Johansson<sup>1,3</sup>, P.R. Njølstad<sup>1,2</sup><sup>1</sup>Department of Clinical Medicine, University of Bergen<sup>2</sup>Department of Pediatrics, Haukeland University Hospital, <sup>3</sup>Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, <sup>4</sup>The Gade Institute, University of Bergen, <sup>5</sup>Department of Pathology, Haukeland University Hospital, Bergen, Norway.

**Background and aims:** Despite successes in identifying genetic causes for hyperinsulinemic hypoglycemia of infancy (HI), only part of the heritable component of this disease has thus far been explained. We hypothesized that copy number variation (CNV) may be responsible for some of the unexplained variation and screened a selected patient material for CNVs.

**Material and methods:** We screened nine subjects from the Norwegian Hypoglycaemia Registry for possible CNVs. The subjects were negative for mutations in the ABCG8, KCNJ11, HADH, HNF4A and GCK genes. Affymetrix 6.0 microarray was used in the CNV screening. Deletions were checked against normal variants generated from 1100 subjects. We collected blood samples and clinical information from members of affected families to look for segregation between CNVs and hypoglycaemia.

**Results:** We identified a duplication on chromosome 1 containing the complete SLC16A1 gene in one family. This duplication was not found in our list of normal variants. Information was obtained from 27 family members in three generations. Blood samples were obtained from 10 family members. In the third generation, 4 children had observed episodes of hypoglycaemia, one also with hyperinsulinemic hypoglycaemia. Neither of the children needed medical intervention and symptoms seem to weaken with increasing age. All had duplication of SLC16A1. In the second generation no episode of hypoglycaemia had been observed. All affected and their parents had increased birth weight, mean 4350g. No family members reported histories of exercise-induced hypoglycaemia.

**Conclusion:** SLC16A1 encodes the Monocarboxylate Transporter 1 and failed silencing of this molecule in pancreatic beta cells has been linked to physical exercise-induced hypoglycaemia. We found a duplication of SLC16A1 in a family with mild hyperinsulinemic hypoglycaemia. The birth weight was increased in affected family members indicating prenatal hyperinsulinism. There was, however, no evidence of exercise-induced hypoglycaemia. This indicates a different molecular mechanism involved in this family by which increased gene dosage might lead to a mild hyperinsulinemic hypoglycaemia only.

Supported by: University of Bergen, Research Council of Norway and Helse Vest

## 626

**Increased risk of severe hypoglycaemic events with increasing frequency of non-severe hypoglycaemic events in patients with type 1 and type 2 diabetes**S. Sreenan<sup>1</sup>, M. Andersen<sup>2</sup>, B.L. Thorsted<sup>2</sup>, M.L. Wolden<sup>2</sup>, M. Evans<sup>3</sup>,<sup>1</sup>Department of Endocrinology, Connolly Hospital, Dublin, Ireland, <sup>2</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>3</sup>Llandough hospital, Cardiff, UK.

**Background and aims:** Severe hypoglycaemic events (SHE) are associated with significant morbidity and mortality, and resultant costs. However, non-severe hypoglycaemic events (NSHE) - despite being more common than SHE - have been less explored, primarily due to an inherent difficulty in measuring their frequency and impact in the real world situation. The aim of this analysis was to investigate the association between reported rates of NSHE and SHE among patients with type 1 (T1DM) and type 2 (T2DM) diabetes mellitus enrolled in the PREDICTIVE study, to establish the odds ratio of experiencing a SHE based on frequency of NSHE.

**Materials and methods:** PREDICTIVE was a global, prospective, observational study. Patients with T1DM (n=7,420) or T2DM (n=12,981), starting treatment with insulin detemir, reported the number of NSHE and SHE experienced during the 4 weeks prior to baseline and follow-up visits (mean 14.4 weeks). SHE were defined as an episode with symptoms of neuroglycopenia, in which the patient was unable to treat himself/herself and third-party intervention was needed, and where the patient had one of the following characteristics: (i) blood glucose <2.8 mmol/L (<50 mg/dL) or (ii) reversal of symptoms after food intake, glucagon or intravenous glucose administration. Confirmed hypoglycaemic events where third-party assistance was not

required were classified as NSHE. Hypoglycaemic events were classified by severity and frequency (NSHE: 0, 1–4, ≥5) (SHE: 0, vs. ≥1). Chi-Square test was used to investigate the link between NSHE and SHE. Logistic regression was used to determine the odds ratio of experiencing ≥1 SHE, in patients having 1–4 or ≥5 NSHE versus those having 0. The logistic regression model controlled for baseline covariates.

**Results:** Baseline demographics (means ±SD) were age, 41.4 ± 16.8 years, duration of diabetes 16.4 ± 12.5 years, BMI 25.2 ± 4.5 kg/m<sup>2</sup> for T1DM, and, 60.6 ± 10.8 years, 11.2 ± 7.5 years and 30.9 ± 5.9 kg/m<sup>2</sup> for T2DM. HbA<sub>1c</sub> at baseline was 8.3 ± 1.7% and 8.6 ± 1.6% in patients with T1DM and T2DM respectively. At follow-up, overall rates of hypoglycaemia were significantly lower for both patients with T1DM and T2DM initiating insulin detemir compared with baseline (*p*<0.0001). There was a statistically significant association between the frequency of NSHE and the experience of SHE in patients with T1DM and T2DM, respectively, at both baseline and follow-up (*p*<0.0001). The odds ratio of experiencing ≥1 SHE increased with frequency of NSHE, for both T1DM and T2DM patients (Table).

**Conclusion:** These observational data, in a large cohort of people with diabetes, confirm a significant association between the frequency of NSHE and subsequent SHE, providing both clinical and economic rationale for the reduction of hypoglycaemic events regardless of severity. Future analyses should aim to define the factors that explain the relationship between NSHE and SHE.

Group/time point	Number of NSHE	Number of patients	Odds ratio of experiencing ≥1 SHE, 95% CI	
T1DM: Baseline n=7,399	0	3,224	1	–
	1–4	2,457	2.07	1.72, 2.49
	≥5	1,718	2.31	1.87, 2.82
T2DM: Baseline n=12,966	0	10,659	1	–
	1–4	1,818	11.10	8.91, 13.81
	≥5	489	15.36	11.47, 20.58
T1DM: Follow-up n=6,837	0	4,190	1	–
	1–4	1,895	2.21	1.51, 3.22
	≥5	752	3.78	2.47, 5.80
T2DM: Follow-up n=12,368	0	11,322	1	–
	1–4	912	16.65	8.66, 32.02
	≥5	134	21.74	7.17, 65.91

CI, Confidence interval; NSHE, non-severe hypoglycaemic event; SHE, severe hypoglycaemic event; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus

Clinical Trial Registration Number: NCT00659295

Supported by: Novo Nordisk

## 627

**Reduction of hypoglycaemia with insulin pump suspension and role of antecedent hypoglycaemia on future hypoglycaemic inductions: ASPIRE study**S.K. Garg<sup>1</sup>, R.L. Brazg<sup>2</sup>, T.S. Bailey<sup>3</sup>, B.A. Buckingham<sup>4</sup>, D.C. Klonoff<sup>5</sup>, J. Shin<sup>6</sup>, J.B. Welsh<sup>6</sup>, F.R. Kaufman<sup>6</sup>,<sup>1</sup>Barbara Davis Center, University of Colorado Denver, Aurora, USA,<sup>2</sup>Rainier Clinical Research Center, Renton, USA, <sup>3</sup>AMCR Institute, Inc.,Escondido, USA, <sup>4</sup>Stanford University, Palo Alto, USA, <sup>5</sup>San Mateo, Mills-Peninsula Health Services, San Mateo, USA, <sup>6</sup>Medtronic, Inc., Northridge, USA.

**Background and aims:** Automatic insulin pump suspension in response to sensor-detected low glucose value may safely and effectively reduce hypoglycemia. The ASPIRE study examined the utility of the low glucose suspend (LGS) feature in hypoglycemia induced by overnight fasting and exercise and the effect of the order of experiments on the duration of hypoglycemia.

**Materials and methods:** Fifty insulin pump users (56% male, mean [±SD] age 34.3±12.4 yr, BMI 26.9±4.3 kg/m<sup>2</sup>, A1C 7.9±0.6%) exercised after an overnight fast until their YSI glucose value was <85 mg/dL with LGS-ON (set to suspend insulin for 2 hours at sensor glucose values ≤70 mg/dL) and with LGS-OFF. Subjects were randomly assigned to Group A (LGS-ON first) or



Group B (LGS-OFF first). A successful experiment was defined by YSI glucose values 50 mg/dL with an observation period of up to 4 hours that was not interrupted by symptomatic hypoglycemia or protocol deviations. After a successful experiment and a washout period of at least 3 days, subjects repeated the experiment with the opposite LGS setting.

**Results:** Of the 134 experiments, 98 were successful (48 LGS-ON and 50 LGS-OFF). Of the 36 unsuccessful experiments, 17 were due to YSI values falling below 50 mg/dL, 14 were due to YSI values not reaching <70 mg/dL, and 5 were due to protocol deviations. The mean duration of hypoglycemia in the 98 successful experiments was significantly less with LGS-ON compared to LGS-OFF ( $138.5 \pm 76.68$  vs.  $170.7 \pm 75.91$  min,  $p=0.006$ ). Mean nadir ( $59.5 \pm 5.72$  vs.  $57.6 \pm 5.69$  mg/dL,  $p=0.015$ ) and mean end-observation YSI glucose values ( $91.4 \pm 41.84$  vs.  $66.2 \pm 13.48$  mg/dL,  $p<0.001$ ) were both higher with LGS-ON than with LGS-OFF. The duration of hypoglycemia in successful LGS-ON experiments was 63.7 min less for Group A than for group B ( $p<0.01$ ). The number of inductions prior to a successful LGS-ON experiment was lower for Group A than for group B ( $0.36 \pm 0.64$  vs.  $1.57 \pm 0.84$ ,  $p<0.001$ ), as was the cumulative duration of antecedent hypoglycemia (Table). The difference in hypoglycemia duration between Groups A and B was not attributable to changes in sensor glucose rates of change, the duration of exercise, or  $AUC<70$  mg/dl in the 2 days before the successful induction (all  $p>0.3$ ).

**Conclusion:** The ASPIRE study showed that automatic insulin pump suspension reduces the duration and severity of hypoglycemia induced by overnight fasting and exercise. Its crossover design highlights that recent antecedent hypoglycemia significantly increases subsequent induced hypoglycemia duration.

Table. Hypoglycemia in the ASPIRE study

	Prior Cumulative Hypoglycemia Duration, min	Hypoglycemia Duration in the LGS-ON Experiment, min
Group A (n=25)	$16.6 \pm 57.3$	$107.8 \pm 71.2$
Group B (n=23)	$204.6 \pm 123.7$	$171.5 \pm 67.0$
p value	<0.001	0.003

Hypoglycemia (defined as YSI <70 mg/dL) duration before and during successful LGS-ON experiments in the in-clinic ASPIRE study (mean  $\pm$  SD)

Clinical Trial Registration Number: NCT01148862

## 628

### Hospitalisations due to severe hypoglycaemia in patients with type 2 diabetes: a US national perspective

G. Singh<sup>1</sup>, A. Mithal<sup>2</sup>, A. Mannalithara<sup>1</sup>, A. Sehgal<sup>3</sup>, M. Bron<sup>4</sup>, O. Dabbous<sup>4</sup>, G. Triadafilopoulos<sup>1</sup>

<sup>1</sup>Stanford University, Palo Alto, <sup>2</sup>Institute of Clinical Outcomes Research and Education, Woodside, <sup>3</sup>University of California, Berkeley, <sup>4</sup>Takeda Pharmaceuticals North America, Inc., Deerfield, USA.

**Background and aims:** Hypoglycemia is a major limiting factor in intensive glycemic control for both type 1 and type 2 diabetes. Severe hypoglycemia is associated with increased risk of adverse clinical outcomes, including cardiovascular complications and possibly increased mortality. We examined the prevalence of serious hypoglycemia hospitalizations in patients with Type 2 diabetes in the US community population.

**Materials and methods:** The Nationwide Inpatient Sample (NIS) is a stratified random sample of all US community hospitals. It is the largest inpatient care database in the US and the only database that has information on all inpatient care regardless of insurance status. NIS's large sample size and data sampling techniques allow calculation of national estimates for particular diagnoses and analysis of secular trends. We studied all inpatient hospitalizations in NIS in 2009 with a primary or secondary diagnosis of Type 2 diabetes and hypoglycemia in patients aged 18 years or older and analyzed them as a proportion of total US resident adult population. US population estimates and projections for the resident US population were obtained from the US Census Bureau.

**Results:** In 2009, there were 33.1 million all-cause hospitalizations in the US among patients 18 years or older in 232.5 million person-years of observation. Of these, 7.2 million (21.7%) hospitalizations were for a primary or secondary diagnosis of Type 2 diabetes, providing a rate of 3,096.8 per 100,000 person-years in the US population. In 2009, there were 248,422 hypoglycemia

hospitalizations in type 2 diabetes patients (average age 67.3 years 95% CIs, 50.7% men), accounting for 3.45% of all hospitalizations in these patients (national prevalence rate 106.9 per 100,000 person-years). Hypoglycemia hospitalizations among type 2 diabetes patients resulted in 1.9 million hospitalization-days in 2009 (average per hospitalization 7.58 days, 95% confidence intervals 7.38 - 7.78), at a total cost of \$12.07 billion (average \$48,569 per hospitalization, 95% confidence limits \$45,781 - \$51,537 per day). Medicare and Medicaid programs were responsible for 76.1 % of these hospitalizations and costs. A large percentage of these hospitalizations (87.5%) were considered non-elective. The case-fatality rate was 3.7%, resulting in 9,274 deaths in patients with hypoglycemia and type 2 diabetes in 2009.

**Conclusion:** National US population-level data suggest that hypoglycemia in Type 2 diabetes was associated with 248,422 hospitalizations and 9,274 deaths in 2009, and a total cost of over \$12 billion. These numbers do not include the long-term indirect clinical consequences of hypoglycemia. While aggressive euglycemic control remains important, the clinical and financial implications of severe hypoglycemia are considerable. Careful selection of anti-diabetic drugs, and close monitoring should be considered to reduce the risk of severe hypoglycemia.

Supported by: Takeda Pharmaceuticals International, Inc.

## PS 043 Regulation of fatty acid metabolism

629

### Novel metabolic associations with polymorphisms in the nicotinic acid receptor

L. Chamas, M. Neville, F. Karpe;  
OCDEM, University of Oxford, UK.

**Background and aims:** *HCAR2* is the endogenous receptor for nicotinic acid (NA), a potent plasma lipid and lipoprotein modifying drug with potential anti-inflammatory properties that conveys an anti-lipolytic signal in adipose tissue. Homology between this receptor and *HCA1* and *HCA3*, another two G-protein coupled proteins that have arisen by homologous duplication, has led to ambiguous characterization of common genetic variation in the region. We aimed to re-sequence *HCA2* and *HCA3* to uncover missing or mislabelled polymorphisms.

**Materials and methods:** Sixty-four subjects were selected from the Oxford Biobank (<http://www.oxfordbiobank.org.uk/>) with equal gender representation. Long range PCR products were created for each of the receptors from genomic DNA. Specificity of the near-identical gene products was tested by a unique base pair difference in one of the genes. Targeted and specific sequencing of these genes beyond the coding region was undertaken on shorter sequences created from the long product. Identified SNPs from the sequencing effort were genotyped on a larger cohort (n=3,500). Expression analysis of the two genes was also performed from adipose tissue paired biopsies in abdominal and gluteal depots (n=200). Data was analyzed and related to anthropometric and metabolic variables from the Oxford Biobank.

**Results:** Five of the existing SNPs in the dbSNP database were found to be referring to the wrong gene. Eight previously unreported SNPs were detected by resequencing. One SNP in the promoter region of *HCA3* affected gene expression of both genes. There was a difference in *HCA2* gene expression in adipose tissue between genders, depots and BMI groups. Gene expression also negatively correlated with waist circumference ( $r=-0.40$ ,  $p<0.001$ ) and positively with HDL cholesterol ( $r=0.34$ ,  $p<0.001$ ). A new SNP in the *HCA2* promoter showed an association with waist to hip ratio in men (n=1,900,  $p=0.011$ ), a finding that will need confirmation in a larger cohort.

**Conclusion:** Resequencing of *HCA2* and *HCA3* revealed a number of mislabeled and new genetic variants with potentially interesting phenotypic associations relevant to the putative gene function.

Supported by: EFSD Clinical research Grant, Rhodes Trust, NDM

630

### Free fatty acids as possible biomarkers in control and progression of prediabetes to type 2 diabetes

S. Mandal<sup>1</sup>, A. Causevic<sup>2</sup>, M. Malenica<sup>2</sup>, T. Dujic<sup>2</sup>, T. Bego<sup>2</sup>, B. Prnjavorac<sup>3,4</sup>, S. Semiz<sup>2</sup>,

<sup>1</sup>Department for Analytical Chemistry I and II, Faculty of Pharmacy, University of Sarajevo, <sup>2</sup>Department for Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, <sup>3</sup>Faculty of Pharmacy, University of Sarajevo, <sup>4</sup>General Hospital Tesanj, Tesanj, Bosnia and Herzegovina.

**Background and aims:** High prevalence of type 2 diabetes (T2D) and obesity represents today a global health problem. The fundamental pathophysiological mechanisms related to T2D include insulin resistance and the decline in pancreatic B-cell function that accompanies an increase in visceral obesity. This is followed by the rise in the plasma concentrations of free fatty acids (FFAs), which are associated with an increase in fat mass and insulin resistance. Numerous studies indicate that FFAs may be potential biomarkers and pharmacological targets in T2D management. In this study we have analyzed concentration of FFAs in patients with T2D and prediabetes, in the context of an optimal diabetes control and monitoring.

**Materials and methods:** The study involved 42 healthy subjects, 46 patients diagnosed with prediabetes, and 73 patients with diagnosed T2D. Diabetic patients were recruited at our hospitals. Preparation of samples for fatty acid analysis was done by using a modified protocol of Le Page and Roy, which involved methanolysis of samples at 50°C for 90 minutes, followed by conversion of FFAs to corresponding fatty acid methyl esters. Detection and quantification of FFAs concentration was done by gas chromatography/mass

spectrometry. In total, 19 FFAs were detected with this protocol. All other biochemical analyses, including glucose, glycosylated hemoglobin (HbA1c), cholesterol, and triglycerides levels, were performed by employing standard IFCC methods.

**Results:** Interestingly, differences in levels of individual FFAs, namely C16:0 were evident between T2D patients and control subjects and C14:0 between prediabetic and T2D patients. Our data showed a significant difference in C16:0 FFA levels between patients with poorly and well-controlled diabetes. There was a significant correlation between HbA1c and C16:0 and C18:1 FFA levels in patients with an adequate T2D control, as well as between HbA1c and C16:0 levels in patients with poor control of the disease.

**Conclusion:** Thus, our data suggest that C16:0, C16:1, and C18:1 could be employed as potential biomarkers in progression and optimal control of T2D, while C14:0, C14:1, C16:0, and C16:1 FFAs could be used as potentially relevant biomarkers in prediabetes.

631

### <sup>13</sup>C-labelled palmitate and metabolomics/lipidomics analyses reveal the fate of free fatty acids in fasting mice

M. Hoene<sup>1,2</sup>, S. Chen<sup>3</sup>, J. Li<sup>3</sup>, E. Schleicher<sup>1,2</sup>, H.-U. Häring<sup>1,2</sup>, G. Xu<sup>3</sup>, C. Weigert<sup>1,2</sup>, R. Lehmann<sup>1,2</sup>;

<sup>1</sup>Internal Medicine IV, Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry, University Hospital Tuebingen, Germany, <sup>2</sup>Paul Langerhans Institute Tuebingen, Member of the German Diabetes Centre, Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Centre Munich at the University of Tuebingen, Tuebingen, Germany, <sup>3</sup>CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China.

**Background and aims:** During fasting, free fatty acids (FFA) released from adipose triglyceride stores become the major source of energy. Because lipolysis is in excess over utilization, plasma FFA are considerably elevated despite an increased utilization by skeletal muscle and other peripheral tissues. The plasma concentration of acylcarnitines, intermediates of fatty acid oxidation, also increases during fasting and exercise, but it is unclear if they originate from skeletal muscle or from the liver, the key metabolic regulator and short-term buffer for excess circulating FFA. Using a stable <sup>13</sup>C-isotope labelled fatty acid tracer in mice, we tested the hypothesis that excess palmitate is incorporated into acylcarnitines in the muscle and into triglycerides and other lipids in the liver in the fasted state.

**Materials and methods:** Male C57BL/6N mice were fasted for 15 h. A bolus of 20 nmol/kg body weight of [U-<sup>13</sup>C]-palmitate was applied into the caudal vein of the anaesthetised mice. After 10 minutes, plasma, liver and gastrocnemius muscles were obtained. Lipids, acylcarnitines and free fatty acids were extracted and analyzed by UPLC-mass spectrometry for [U-<sup>13</sup>C]-palmitate-derived metabolites. Values are means ± SD from n=7 mice.

**Results:** The amount of [U-<sup>13</sup>C]-palmitate injected was aimed to correspond to the increase in plasma FFA typically caused by 15 h fasting or 1 h of moderately intense running in mice, to a calculated maximal plasma concentration of 300 μmol/L. Ten minutes after the bolus injection, 2.5±0.5 μmol/L free tracer were left in plasma and 39±12 and 14±4 nmol/g<sub>protein</sub> in liver and muscle, respectively. Acylcarnitines derived from the tracer reached a plasma concentration of 0.82±0.18 nmol/L and were considerably higher in muscle than in the liver, 0.95±0.47 versus 0.002±0.001 nmol/g<sub>protein</sub>. Lipids incorporating palmitate tracer were only detectable in the liver, a total of 511±160 nmol/g<sub>protein</sub> as triglycerides and 58±9 nmol/g<sub>protein</sub> as phosphatidylcholine.

**Conclusion:** By using <sup>13</sup>C-labelled palmitate as a tracer, we could show that compared to skeletal muscle, the production of acylcarnitines from long-chain FFA in the liver is negligible. Thus, it can be concluded that the muscle and not the liver is responsible for the increase in plasma long-chain acylcarnitines during fasting. In addition, this tracer study confirmed the central role of the liver as buffering system for the storage of excess fatty acids present in the circulation during fasting and revealed that the bulk of labelled palmitate is incorporated into hepatic triglyceride and phosphatidylcholine.

Supported by: DFG grant to CW, BMBF for DZD e.V.

## 632

**A natural compound FST6 regulates glucose and lipid metabolism in adipocytes by inhibiting 11 beta- HSD1 and enhancing AMPK signalling pathway**

S. Huang, H. Zeng, M. Ning, Y. Leng;

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.

**Background and aims:** 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) and AMP-activated protein kinase (AMPK) are attractive therapeutic targets of type 2 diabetes and metabolic syndrome. FST6, a nature compound was discovered from our natural products library as a potent 11 $\beta$ -HSD inhibitor, and it could also active AMPK signaling pathway. In the present study, the effects of FST6 on glucose and lipid metabolism in adipocytes were investigated and its possible mechanisms were explored.

**Materials and methods:** Scintillation proximity assay was performed to evaluate inhibition of FST6 against 11 $\beta$ -HSDs from recombinant human and mouse 11 $\beta$ -HSDs. Its 11 $\beta$ -HSD1 inhibitory activity *in vivo* was investigated in C57BL/6J mice. Activation of FST6 on AMPK signaling pathway was investigated by western blot analysis. Glucose uptake and fatty acid oxidation in 3T3-L1 adipocytes were measured. 11-DHC (inactive glucocorticoids), corticosterone (active glucocorticoids) and compound C were used for mechanism exploration.

**Results:** FST6 is a potent and selective 11 $\beta$ -HSD1 inhibitor with the IC<sub>50</sub> of 58.6nM and 17.8nM for human and mouse 11 $\beta$ -HSD1. It also inhibited 11 $\beta$ -HSD1 activity in 3T3-L1 adipocytes with the IC<sub>50</sub> of 2.1 $\mu$ M. Single oral administration of FST6 reduced 11 $\beta$ -HSD1 activity to 67.8% in mesenteric adipose tissue of C57BL/6J mice. FST6 increased AMPK and ACC phosphorylation significantly in 3T3-L1 adipocytes in a dose dependent manner. Moreover, single oral administration of FST6 at dose of 300 mg/kg in C57BL/6J mice increased AMPK and ACC phosphorylation in mesenteric adipose tissue. Both 11-DHC (inactive glucocorticoids) and corticosterone (active glucocorticoids) attenuated insulin stimulated glucose uptake in 3T3-L1 adipocytes. FST6 significantly reversed the impaired insulin stimulated glucose uptake induced by 11-DHC, but not corticosterone. FST6 dose dependently increased free fatty acid oxidation in 3T3-L1 adipocytes after 2 h treatment. This effect could be completely blocked by the pretreatment of Compound C, an AMPK inhibitor.

**Conclusion:** Our results demonstrate that FST6 is a potent and selective 11 $\beta$ -HSD1 inhibitor and activates AMPK simultaneously, which regulates glucose and lipid metabolism in adipocytes. These findings suggest that FST6 may have beneficial effects in the treatment of adipose abnormality in type 2 diabetes and metabolic syndrome.

## 633

**Unravelling eicosanoid action on lipid metabolism and glucose homeostasis during obesity**S. Franckhauser<sup>1,2</sup>, I. Elias<sup>1,2</sup>, T. Ferré<sup>1,2</sup>, S. Muñoz<sup>1,2</sup>, M. Garcia<sup>1,2</sup>, J. Agudo<sup>1,2</sup>, F. Bosch<sup>1,2</sup>;<sup>1</sup>Center of Animal Biotechnology and Gene Therapy, Universitat Autònoma de Barcelona, Bellaterra, <sup>2</sup>CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain.

**Background and aims:** Adipose tissue macrophage infiltration and inflammatory cytokine secretion may play a role in the onset of insulin resistance and atherosclerosis during obesity. Recently, eicosanoids have been described to play an important role in adipose tissue inflammation. It has been observed that expression of arachidonate 5-lipoxygenase-activating protein (Alox5ap) gene, coding for a key protein in eicosanoid synthesis, is increased in adipocytes from human patients with obesity and insulin resistance, and in adipose tissue from obese and insulin resistant mice.

**Material and methods:** Thus, to study the effects of an increase in Alox5ap expression in adipocytes, transgenic mice over-expressing Alox5ap in adipose tissue were generated and characterized.

**Results:** Two transgenic lines were obtained, in which mRNA Alox5ap levels were increased in white and brown adipose tissue. This was parallel to a slight increase in LTB<sub>4</sub> and MCP-1 synthesis in WAT explants from both transgenic lines compared with controls. Moreover, transgenic mice presented decreased body weight and length than control mice and a lower fat pad weight. This was associated with an increase in energy expenditure. Transgenic mice were glucose intolerant, without presenting whole-body insulin resistance, accordingly to a defect in glucose-induced insulin secretion. When challenged a high fat diet, these mice gained less body weight, but similar fat mass and

they remained more insulin sensitive than control mice. This was associated with decreased circulating levels of insulin, free fatty acids, triglycerides and glycerol. In contrast, serum total cholesterol and HDL-cholesterol levels were higher in transgenic mice. In addition, the expression of inflammation markers in adipose tissue was reduced in these mice.

**Conclusion:** These results suggest that Alox5ap overexpression in adipose tissue does not lead to insulin resistance but to defects in insulin secretion and may protect against high fat diet-induced inflammation and insulin resistance.

*Supported by: Instituto de Salud Carlos III (PI 070313), CIBERDEM (ISCIII, Ministerio)*

## 634

**Role of bombesin receptor subtype-3 and its agonist [D-Phe<sup>6</sup>- $\beta$ -Ala<sup>11</sup>,Phe<sup>13</sup>,Nle<sup>14</sup>]bombesin<sub>6-14</sub> in lipid metabolism**Z. Moreno-Villegas<sup>1</sup>, I. Ramos-Álvarez<sup>2</sup>, R. Sanz<sup>1</sup>, R. Jensen<sup>3</sup>, N. González<sup>2</sup>;<sup>1</sup>Metabolism, Nutrition and Hormones, IIS- Fundación Jiménez Díaz,Madrid, Spain, <sup>2</sup>IIS- Fundación Jiménez Díaz, CIBERDEM, Madrid, Spain,<sup>3</sup>National Institutes of Health, Bethesda, USA.

**Background and aims:** Bombesin Receptor Subtype BRS-3 receptor deficient mice develop a mild-late-onset obesity with metabolic alterations, which could be due to impairment in the glucotransporter-4 (glut-4) translocation mechanism in adipocytes. Moreover, we described that BRS-3 gene expression is lower than normal in skeletal muscle of diabetic and/or obese patients and also, in primary cultured myocytes from normal or altered metabolic patients, agonist [D-Phe<sup>6</sup>- $\beta$ -Ala<sup>11</sup>,Phe<sup>13</sup>,Nle<sup>14</sup>]bombesin<sub>6-14</sub> (BRS-3-AP) stimulated glucose transport, glut-4 and BRS-3 expression, kinase phosphorylation level (PKB, MAPKs, p70s6K), PI3K activity and glucose metabolism. Here, in rat adipose tissue without metabolic alterations, we have studied BRS-3 gene expression, characteristics of the receptor signalling pathways by using BRS-3-AP, and explored its effect on lipid metabolism.

**Methods:** Adipocytes were isolated by enzymatic digestion from epididymal fat pads of 10 normal male Wistar rats (200–250 g), kept in a standard chow and water ad libitum. We measured: BRS-3 gene expression -real time-PCR-, MAPKs and p90RSK-1 activity -Western Blot-, lipogenesis as <sup>14</sup>C-Na acetate incorporation into lipids, in cells incubated in BRS-3-AP absence (control) or presence (10<sup>-13</sup>-10<sup>-7</sup> M), and with or without 10<sup>-6</sup> M wortmanin -PI3K/ PKB inhibitor-, 2.5x10<sup>-5</sup> M PD98059 -MAPKs inhibitor-, 10<sup>-7</sup> M rapamycin -p70s6K inhibitor-.

**Results:** We demonstrated by RT-PCR the presence of BRS-3 mRNA, in rat adipose tissue. The synthetic agonist BRS-3-AP caused a lipogenesis concentration-related stimulation, already detected at 10<sup>-11</sup> M (118 $\pm$ 6% control), which achieved significance values (10<sup>-10</sup> M: 128 $\pm$ 7% control, *p*<0.05; 10<sup>-9</sup> M: 136 $\pm$ 5% and 10<sup>-8</sup> M: 168 $\pm$ 18%, *p*<0.02) and was maximal at 10<sup>-7</sup> M (209 $\pm$ 20% control, *p*<0.02). The lipogenic effect of BRS-3-AP (10<sup>-8</sup> M: 164 $\pm$ 13% control, *p*<0.01), not different from that induced by insulin (10<sup>-8</sup> M: 140 $\pm$ 9% control, *p*<0.01) was abolished by the additional presence of wortmanin (108 $\pm$ 5% control) and the same blocking effect was detected in the presence of PD-98059 (104 $\pm$ 11% control). However, rapamycin only partially reduced the effect of the synthetic BRS-3 ligand (113 $\pm$ 5% control, *p*<0.05 vs 10<sup>-8</sup> M BRS-3-AP). BRS-3-AP at 10<sup>-9</sup> M clearly increased (*p*<0.05) MAPKs phosphorylation (p44: 141 $\pm$ 11% control; p42: 230 $\pm$ 40%; *p*<0.05 or lower) like it did 10<sup>-9</sup> M insulin (p44: 144 $\pm$ 9% control; p42: 146 $\pm$ 20%; *p*<0.05). In addition, p90RSK-1 phosphorylation level was significantly (*p*<0.05) increased by the synthetic agonist at 10<sup>-9</sup> M and 10<sup>-8</sup> M (169 $\pm$ 5% control and 171 $\pm$ 21% control, respectively), similar to that induced by 10<sup>-9</sup> M insulin (153 $\pm$ 11% control, *p*>0.05).

**Conclusion:** These results reveal a specific role of BRS-3 in adipose tissue -potential origin of insulin-resistance- and not only describe the lipogenic effect of its synthetic agonist but also, clarify the receptor signalling pathway. These facts points out the receptor, and/or its agonist peptide, as a molecular target in the therapy of diabetes and obesity.

*Supported by: FIS, CIBERDEM, ISC III and Fundación Rodríguez Pascual, Spain*



## PS 044 Mechanisms of NAFLD progression

635

### Molecular insights of liver steatosis and inflammation in db/db mice

M.F. Scheerer<sup>1,2</sup>, B. Fridrich<sup>1</sup>, G. Kastenmüller<sup>3</sup>, M. Irmeler<sup>1</sup>, M. Kahle<sup>1</sup>, J. Beckers<sup>1</sup>, K. Suhre<sup>3,4</sup>, H.-W. Mewes<sup>3</sup>, M. Hrabé de Angelis<sup>1,2</sup>, S. Neschen<sup>1,2</sup>; <sup>1</sup>Institute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg/Munich, Germany, <sup>2</sup>German Mouse Clinic, Helmholtz Zentrum München, Neuherberg/Munich, Germany, <sup>3</sup>Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg/Munich, Germany, <sup>4</sup>Bioinformatics Core, Weill Cornell Medical College, Doha, Qatar.

**Background and aims:** Leptin receptor (LR) gene polymorphisms have been shown to contribute to the onset of Non-Alcoholic Fatty Liver Disease (NAFLD) in rodents and humans. Aim of the study was to examine the role of a non-functional leptin receptor in a db/db mouse model in the pathophysiology of hepatic steatosis.

**Materials and methods:** BKS.Cg + Leprdb/+ Leprdb (db/db) and BKS.Cg Dock7m +/Dock7m + (wt) mice (n=10 each group) were fed a high fat diet after weaning at an age of three weeks. At 10 weeks of age, mice were fasted for four hours and plasma and organs dissected under isoflurane anesthesia. Besides plasma parameters characterizing organ function, glucose and lipid homeostasis, we compared hepatic gene expression (Illumina) as well as hepatic and plasma metabolite profiles via a non-targeted approach (Metabolon, LC-MS, GC-MS) in both genotypes.

**Results:** After seven weeks of high fat diet feeding, obese db/db mice displayed a pronounced diabetic phenotype compared to lean, wild type controls (db/db vs. WT; HbA1c: 57±7 vs. 21±1 mmol/mol; plasma glucose: 43.1±2.2 vs. 17.0±1.8 mmol/l, plasma insulin: 791±350 vs. 85±11 mol/l). Elevations in plasma CRP (db/db vs. WT; 13.3±1.7 vs. 5.4±0.8 mg/l), triglycerides (db/db vs. WT; 2.6±0.4 vs. 1.3±0.3 mmol/l) and ALT concentrations (db/db vs. WT: 130±51 vs. 29±4 U/l) paralleled pronounced hepatosteatosis (db/db vs. WT; liver triacylglycerol: 1652±149 vs. 295±27 μmol/g). An increase in homocysteine concentrations in presence of decreasing methionine concentrations was detected in db/db mouse livers. Such an alteration in the methionine:homocysteine ratio could impair S-adenosylmethionine (SAM) cycle activity what might result in an increased availability of educts for the rate limiting step of glutathione synthesis - the conversion from cysteine and glutamate to glutamylcysteine. Interestingly, the hepatic transcript of the catalytic subunit of glutamate-cysteine ligase was 3-fold downregulated. This could potentially alter the GSH/GSSG ratio in db/db mouse livers and contribute to oxidative stress and inflammation in hepatosteatosis.

**Conclusion:** A decrease in SAM in liver has been implicated in the initial steps of hepatic steatosis in db/db mice raised on a methionine deficient diet. However, our findings show increased inflammation even when SAM levels remain unaffected. Solely hepatic glutathione concentrations - in absence of alterations in pro-inflammatory arachidonic acid derivative levels - were changed in NAFLD. Thus, our findings indicate that progression from benign to malign NAFLD might depend on a reduced glutathione production rate in mice with impaired signalling through the leptin receptor.

*Supported by: SysMBO, German BMBF to the DZD e.V., NGFN-Plus (01GS0850)*

636

### Astaxanthin reduces hepatic insulin resistance and inhibits the progression of lipotoxic model of nonalcoholic steatohepatitis

T. Ota, Y. Ni, L. Zhan, M. Nagashimada, S. Kaneko;  
Frontier Science Organization, Kanazawa University, Japan.

**Background and aims:** Nonalcoholic steatohepatitis (NASH) and insulin resistance frequently coexists in subjects with obesity and type 2 diabetes. NASH can be defined as a lipotoxic liver injury caused by excess body adiposity. Hepatic inflammation and/or fibrosis could be caused by lipid peroxidation preceded by excessive hepatic lipid accumulation. In our previous study, we developed a cholesterol- and saturated fatty acid-induced model of lipotoxic NASH and revealed that hepatic oxidative stress and insulin resistance promotes hepatic inflammation and fibrosis. Astaxanthin is a carotenoid compound that is known to be approximately 500 times more potent in inhibiting lipid peroxidation than vitamin E *in vitro*. In the present study,

we examined the inhibitory effect of astaxanthin against the progression of NASH to clarify the significance of lipid peroxidation-mediated lipotoxicity and insulin resistance in the pathogenesis of NASH.

**Materials and methods:** 7-week-old C57BL/6 mice were fed on a high-cholesterol/high-fat diet (CL) or a CL diet containing 0.02% astaxanthin (CL + Ax), for a total of 12 weeks. The lipid peroxidation, insulin sensitivity, and inflammatory/stress signal were evaluated.

**Results:** After 8 weeks of feeding, the levels of thiobarbituric acid reactive substances (TBARS) in liver were significantly increased and histological examination revealed hepatic steatosis and inflammation. At week 12, hepatic fibrosis was observed in mice fed CL diet. They showed hyperinsulinemia (CL1.4±0.3 vs normal chow (NC) 0.3±0.1 ng/ml,  $p < 0.05$ ) and glucose intolerance even though weight and adiposity were similar, indicating that mice fed on a CL diet developed NASH associated with insulin resistance. Astaxanthin administration markedly reduced lipid peroxidation and improved glucose intolerance and insulin sensitivity in CL group. CL group had 2.5-fold and 4.1-fold increases in hepatic triglyceride (TG) and total cholesterol (TC) levels compared to NC group; astaxanthin decreased hepatic TG and TC levels by 38% and 46%, respectively (all  $p < 0.05$ ). The  $\alpha$ -SMA positive cell, as an index of activated stellate cells, increased in CL group, but decreased significantly in CL+Ax group. Furthermore, CL treatment led to 1.5-fold and 6-fold increase in TGF- $\beta$  and collagen I mRNA expression, and 4.3-fold increase in hydroxyproline content, whereas astaxanthin administration inhibited fibrosis, lowering the TGF- $\beta$  and collagen I mRNA expression, and hydroxyproline content by 28%, 35%, and 24%, respectively (all  $p < 0.05$ ). Astaxanthin administration in CL-fed mice led to enhanced insulin signal assessed by IR $\beta$  and Akt phosphorylation in the liver. These findings were associated with reduction of ER stress (CHOP/GRP78), attenuation of MAPK (JNK/p38MAPK) and NF- $\kappa$ B activation in liver. Astaxanthin, however, showed little effect on insulin resistance and stress response in the adipose tissue in the CL-treated mice.

**Conclusion:** Hepatic cholesterol and TG accumulation causes excessive lipid peroxidation and the development of insulin resistance, leading to hepatic inflammation and fibrosis. Astaxanthin, a strong antioxidant, inhibits the progression of NASH by attenuating lipid accumulation, lipid peroxidation, and insulin resistance.

*Supported by: A-STEP/JST*

637

### Prep1 deficiency is associated with a reduced hepatic lipogenesis and attenuates methionine choline deficient diet-induced steatohepatitis

S. Cabaro<sup>1</sup>, F. Oriente<sup>1</sup>, A. Liotti<sup>1</sup>, L. Parrillo<sup>1</sup>, T.B. Pagano<sup>2</sup>, O. Paciello<sup>2</sup>, F. Blasi<sup>3</sup>, P. Formisano<sup>1</sup>, F. Beguinot<sup>1</sup>;

<sup>1</sup>DBPCM, University of Naples, <sup>2</sup>DPAH, University of Naples, <sup>3</sup>IFOM (FIRC Institute of Molecular Oncology), Milan, Italy.

**Background and aims:** Non-alcoholic steatohepatitis (NASH) is a liver disease frequently associated with the clinical features of metabolic disorders such as insulin-resistance, that causes fat accumulation, inflammation and fibrosis. In fact, several evidences have shown that in presence of insulin-resistance the impairment of the AMPK signaling may be associated with increased lipogenesis and onset of hepatic steatosis. Recent data have suggested the implication of some transcription factors both in insulin-resistance and steatosis. Prep1 is a homeodomain transcription factor belonging to the TALE proteins, which plays an important role in hematopoiesis, organogenesis and development. Previous studies have indicated that Prep1 heterozygous (Prep1<sup>+/+</sup>) mice, which express only 55-57% of protein, have a complex metabolic phenotype with at least two relevant features. One is the presence of smaller but otherwise normally structured islets with reduced fasting and post-loading plasma insulin levels. The second is increased insulin sensitivity in skeletal muscle and in liver which is accompanied by protection from streptozotocin-induced diabetes. In muscle decreased Prep1 levels are followed by an increase of the PGC1 $\alpha$ /Glut4 mediated glucose uptake. In liver, better insulin sensitivity is due to a reduced Shp1 tyrosine phosphatase expression followed by an increase of insulin signaling. In this study we have focused our attention on the role of Prep1 on the regulation of lipogenesis and how this effect could be related to MCD diet-induced NASH.

**Methods and results:** To study the lipogenesis in the liver of the Prep1 heterozygous mice, we have measured triglyceride content both in serum and in liver and hepatic expression of the lipogenic enzyme FAS (Fatty Acid Synthase). Hepatic FAS mRNA levels are significantly decreased. Western Blot analysis have shown increased phosphorylation of PKC $\zeta$ , LKB1, AMPK and ACC, which may control FAS expression and triglycerides production in

Prep1<sup>+/+</sup> mice liver. mRNA and protein levels of the lipid phosphatase SHIP2, an inhibitor of PI3Kinase/PKCzeta signaling, are reduced by 40% in the liver of Prep1<sup>+/+</sup> mice. To understand the potential role of the Prep1-mediated lipogenesis, hepatic steatosis has been induced feeding WT and Prep1<sup>+/+</sup> mice a methionine and choline-deficient (MCD) or a control diet (CD) for 28 days. In Prep1<sup>+/+</sup> mice receiving the MCD diet serum ALT levels are 50% lower than the WT mice. Moreover, hepatic triglyceride content is diminished by 30% at the onset and the outcome of the MCD diet in Prep1<sup>+/+</sup> mice compared to the control littermates, indicating that Prep1 reduction protects in part from hepatic steatosis.

**Conclusion:** All together these data suggest that Prep1-deficiency reduces lipotoxicity by the increasing PKCzeta/AMPK signaling and ameliorates NASH giving a rationale to investigate Prep1 as possible new therapeutic agents in preventing fatty liver progression to NASH.

## 638

### Effect of acute hepatic overexpression of SIRT3 on metabolic parameters in short-term high fat fed mice

B. Osborne, M. Montgomery, J. Reznick, G.J. Cooney, N. Turner; Garvan Institute of Medical Research, Sydney, Australia.

**Background and aims:** Dysregulated mitochondrial metabolism has been linked with several human disorders, including insulin resistance and type 2 diabetes. Recent studies have shown that the sirtuin family of NAD<sup>+</sup>-dependent deacetylase enzymes play an important role in the regulation of mitochondrial function. In particular, SIRT3 has been shown to directly regulate the activity of a range of mitochondrial proteins, suggesting a key role for this enzyme in the regulation of energy metabolism. SIRT3 knockout mice are insulin resistant when maintained on a low-fat chow diet and display accelerated development of the metabolic syndrome when exposed to a long-term high fat diet (HFD). Given the detrimental effects of SIRT3 deletion, the aim of the current study was to determine if hepatic overexpression of SIRT3 could protect against metabolic defects induced by high fat feeding in mice.

**Materials and methods:** C57BL6 mice were subjected to hydrodynamic tail vein injection of a plasmid containing hSIRT3-FLAG, to produce specific overexpression in the liver. A control group was similarly injected with the plasmid encoding the empty vector. Mice were then maintained on either a chow or HFD for 3 weeks and glucose tolerance was assessed using an intraperitoneal glucose tolerance test (2g/kg). Expression of SIRT3 was assessed by real-time PCR and western blot and liver triglycerides were also measured.

**Results:** Liver Sirt3 mRNA expression was increased approximately 9-fold over control ( $4.6 \pm 0.113$  vs.  $39.7 \pm 0.769$  control vs. SIRT3-FLAG,  $p < 0.001$ ). Overexpression at the level of protein was evident by an additional band directly above the endogenous mSIRT3 protein band on a western blot, corresponding to human SIRT3-FLAG (90% increase in total SIRT3 protein by densitometric analysis,  $p < 0.01$ ). At 3 weeks, control mice fed the HFD exhibited a 2.5-fold ( $p < 0.01$ ) increase in the mass of the epididymal fat pad and displayed a significant impairment in glucose tolerance compared to chow-fed animals (~2-fold increase in incremental area under the curve during the glucose tolerance test,  $p < 0.001$ ). Furthermore, liver triglyceride levels were significantly higher in fat-fed mice ( $8.5 \pm 1.37$  vs.  $17.6 \pm 2.25$   $\mu\text{mol/g}$ ,  $p < 0.01$ ). Despite significant increases in expression at both the mRNA and protein level, mice with hepatic overexpression of SIRT3 displayed similar impairments in glucose tolerance and a comparable degree of triglyceride accumulation in response to the HFD as control animals.

**Conclusion:** While previous studies in knockout animals show detrimental metabolic effects, current results suggest that overexpression of the mitochondrial deacetylase enzyme SIRT3 in mouse liver in vivo does not protect against hepatic lipid accumulation and glucose intolerance induced by a short-term HFD. It remains possible that prolonged SIRT3 overexpression may have beneficial metabolic effects in long-term high fat feeding or that other components of the sirtuin pathway (e.g. NAD<sup>+</sup> availability) are critical for sirtuin regulation of metabolism.

## 639

### Both lipid and protein phosphatase activities of PTEN control hepatic gluconeogenesis and lipid storage in the liver

L. Bourgoïn<sup>1</sup>, M. Peyrou<sup>1</sup>, S. Clément<sup>2</sup>, F. Negro<sup>2,3</sup>, M. Foti<sup>1</sup>;

<sup>1</sup>Cell Physiology and Metabolism, University Medical Center, <sup>2</sup>Division of Clinical Pathology, University Medical Center, <sup>3</sup>Division of Gastroenterology and Hepatology, University Hospital, Switzerland.

**Background and aims:** PTEN is one of the most important tumor suppressor identified to date and an important negative regulator of PI3K signaling. It functions as a dual phosphatase being capable of dephosphorylating both phosphoinositides, thereby antagonizing PI3K signaling, and proteins. Liver-specific PTEN knockout mice develop within months the whole spectrum of non-alcoholic fatty liver diseases and hepatocellular carcinoma with ageing, but paradoxically, these mice have an enhanced peripheral glucose tolerance. We further demonstrated that with obesity and the metabolic syndrome, PTEN expression is decreased in the liver of different rat models and in human subjects. This study aims now at understanding the molecular mechanisms by which PTEN deficiency improves systemic glucose tolerance, while promoting aberrant lipid storage in the liver.

**Materials and methods:** 4-month old AlbCre<sup>+/+</sup>/PTEN<sup>flx/flx</sup> mice and littermate controls were subjected to metabolic tests and ex-vivo analyses of liver tissues. In parallel, insulin signaling and the lipid metabolism was investigated in Huh-7 cells transduced with retroviral constructs expressing either shRNAs against PTEN, or dominant negative mutants of PTEN impaired in their lipid or protein phosphatase activities.

**Results:** 4-months old mice lacking PTEN specifically in the liver displayed abnormal ALT/AST serum levels, a marked hepatomegaly (liver weight:  $+75.7 \pm 12\%$ ,  $p < 0.001$ ) and steatosis (liver triglyceride:  $+426.7 \pm 11\%$ ,  $p < 0.001$ ), but an increased peripheral glucose tolerance. Hepatic deficiency of PTEN did not alter the body weight and the ratio of lean vs fat mass, the food/water intake, locomotor activity, thermoregulation, and more generally, energy expenditure. However, these mice were hypoglycemic in fasted state ( $-34.6 \pm 10\%$ ,  $p < 0.5$ ) and had a strong inhibition of gluconeogenesis in response to pyruvate challenge. Inhibition of gluconeogenesis was associated with an increased Akt activity, although insulin receptors and insulin receptor substrates (IRSs) proteins expressions/activities were strongly downregulated. Surprisingly, only inhibition of the protein, but not the lipid, phosphatase activity of PTEN, using dominant negative mutants, was found to induce steatosis in Huh-7 cells. In contrast, Akt activity was only enhanced by mutants impairing the lipid phosphatase activity of PTEN.

**Conclusion:** Inhibition of hepatic gluconeogenesis contributes to the increased glucose tolerance in liver-specific PTEN knockout mice. Whereas the lipid phosphatase activity of PTEN controls Akt activation, the development of steatosis in hepatocytes is associated with deficiency in the protein phosphatase activity of PTEN.

Supported by: FNS to FN; FNS, FRRD and EFSD Diabetes and Cancer grant

## 640

### Deletion of the small heterodimer partner gene protects against fatty liver and dyslipidaemia, but not obesity and glucose intolerance in C57BL/6J mice fed a high-fat diet

K. Iizuka, W. Wudelehu, R. Tomita, H. Tsuchida, Y. Horikawa, J. Takeda; Department of Diabetes and Endocrinology, Gifu university, Japan.

**Background and aims:** The orphan nuclear receptor small heterodimer partner (SHP) has an important role in the regulation of metabolic pathways that are involved in hepatic bile acid production as well as in lipid and glucose homeostasis. However, the role of SHP in metabolic syndrome is not clear. We examined the effects of homozygous SHP knockout (SHP<sup>-/-</sup>) on metabolic syndrome development in C57BL/6J mice. Moreover, we examined whether glucagon-like peptide-1 (GLP-1) analogs affect the metabolic changes in SHP<sup>-/-</sup> mice fed a high-fat diet.

**Materials and methods:** From 13 weeks of age, wild-type (WT) and SHP<sup>-/-</sup> C57BL/6J mice were fed a high-fat diet [Quick Fat diet™ (CLEA Japan, Inc., Tokyo, Japan)] containing 14% fat, 25% protein, and 46.3% starch. At 30 weeks of age, the GLP-1 analog, liraglutide (50  $\mu\text{g/kg}$  body weight/day) or saline (placebo) was administered for 5 days. We examined glucose tolerance, lipid profiles, body weight, histology of liver sections, and mRNA levels.

**Results:** Body weights were similar between WT and SHP<sup>-/-</sup> C57BL/6J mice fed a high-fat diet (WT:  $34.56 \pm 2.89$  g vs SHP<sup>-/-</sup>:  $34.38 \pm 3.43$  g). However, liver weights in SHP<sup>-/-</sup> mice were much lower than those in WT mice (WT:

4.69 ± 0.20% vs SHP-/-: 3.80 ± 0.22%). Blood glucose levels were similar between SHP-/- and WT mice. Interestingly, hepatic fatty changes in histological sections were not detected in SHP-/- mice fed a high-fat diet. Fasting total cholesterol levels in SHP-/- mice were much lower than those in WT mice (WT: 126.18 ± 7.56 mg/dl vs SHP-/-: 81.09 ± 14.27 mg/dl, respectively). In contrast, fasting triglyceride levels in SHP-/- mice were higher than those in WT mice (WT: 98.9 ± 16.41 mg/dl vs SHP-/-: 110.85 ± 9.36 mg/dl). In the livers of SHP-/- mice fed a high-fat diet, SHP mRNA levels were not detected, farnesoid X receptor mRNA levels increased 1.6 times, and peroxisome proliferator-activated receptor-γ (PPARγ) mRNA levels decreased by 40% compared to WT mice fed a high-fat diet. Moreover, cytochrome P450 A1 (Cyp7A1) mRNA levels, by which cholesterol is converted into bile acid, were increased 13 times in SHP-/- mice. Finally, in SHP-/- mice fed a high-fat diet, 5 days of liraglutide administration effectively improved body weight, glucose tolerance, and lipid profiles to levels that were similar to WT mice.

**Conclusion:** Loss of SHP expression protected against high fatty liver and dyslipidemia through decreased expression of PPARγ mRNA and increased expression of Cyp7A1 mRNA. GLP-1 analogs were effective in metabolic syndrome observed in both WT and SHP-/- mice fed a high-fat diet. Combination therapy of SHP inhibitors and GLP-1 analogs may be a potential treatment strategy for metabolic syndrome.

## 641

### Enhanced PPARγ and NF-κB activity contributes to impaired insulin sensitivity and ectopic fat deposition in SIRT1<sup>+/-</sup> mice

F. Xu<sup>1</sup>, Z. Gao<sup>2</sup>, J. Zhang<sup>2</sup>, J. Ye<sup>2</sup>, J. Weng<sup>1</sup>

<sup>1</sup>Endocrinology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China, <sup>2</sup>Antioxidant and Gene Regulation Laboratory, Pennington Biomedical Research Center, Baton Rouge, USA.

**Background and aims:** SIRT1 is a class III histone deacetylase whose activity is activated in the body by fasting or calorie restriction. It was reported that SIRT1 promoted lipid mobilization by inhibition of ligand-dependent PPARγ activity in adipocytes. SIRT1 was also reported to inhibit inflammation by suppressing the transcriptional activity of NF-κB. However, those activities were observed in cellular models. It remains unclear how these SIRT1 activities are integrated in vivo in the regulation of lipid metabolism. To address this issue, we examined metabolic phenotype of the SIRT1<sup>+/-</sup> mice.

**Materials and methods:** The SIRT1 heterozygous KO (SIRT1<sup>+/-</sup>) mice and wild type (WT) littermates with C57BL/6 background were used as subjects in the study. Systemic evaluations were as follows: Fat mass and body composition was measured using quantitative nuclear magnetic resonance (NMR). Blood glucose was measured every 2–4 week. Insulin was detected in the plasma both on basal level and after diet intervention. Intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (IPITT) were performed after 12 weeks diet intervention. Liver and fat tissues (epididymal and retroperitoneal fat pads) were collected and weighed, and histology study was carried out with liver and fat tissue such as HE staining and immunohistochemistry. Quantitative real-time PCR and western blotting were used to detect mRNA and protein expression, respectively.

**Results:** Compared to the wild type littermates, the SIRT1<sup>+/-</sup> mice had more body fat on chow diet. Their lean body mass, physical activity and food intake were not altered. The increased adiposity did not induce adipose tissue expansion as the mass of epididymal fat, retroperitoneal fat, and inguinal fat pads was not increased in the SIRT1<sup>+/-</sup> mice, however, liver weight and liver/body ratio was elevated, suggesting an ectopic fat deposition in the liver. IPGTT and IPITT results showed impaired glucose tolerance and insulin sensitivity in the SIRT1<sup>+/-</sup> mice, respectively. The adipose tissue exhibited an elevation in inflammation as indicated by cytokine expression and macrophage infiltration, suggesting an increase in the NF-κB activity. However, the adipose tissue structure was not altered in adipocyte size, cell density, and the extracellular matrix. In vitro, adipogenesis of SIRT1<sup>+/-</sup> preadipocyte was enhanced as determined by staining with DAPI and BODIPY493/503. Hepatic steatosis was enhanced as indicated by liver/body ratio, liver size, and lipid content, which were all significantly increased in the SIRT1<sup>+/-</sup> mice. Protein expression of liver suggesting enhanced PPARγ and NF-κB activity.

**Conclusion:** In the SIRT1<sup>+/-</sup> mice, the reduced SIRT1 activity leads to impaired insulin sensitivity, but failed to induce adipose tissue expansion. The data suggests that the enhanced NF-κB activity may promote inflammation in adipose tissue and inhibit the tissue expansion. The elevated PPARγ and NF-κB activity in hepatocytes together with the adipose tissue dysfunction may promote the hepatic steatosis. Therefore, the enhanced NF-κB and

PPARγ activity may be the underlying mechanism for the impaired insulin sensitivity and ectopic lipid deposition in the SIRT1<sup>+/-</sup> mice.

*Supported by: PCSIRT(985 project IRT 0947), NIH (Grants DK068036 and DK085495)*

## 642

### Metabolic effects of dietary n-3 fatty acids supplied as phospholipids reflect down-regulation of biosynthetic pathways in the liver of mice fed a high-fat diet

J. Roubalova, M. Rossmeisl, J. Kopecky;

Adipose Tissue Biology, Institute of Physiology Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic.

**Background and aims:** We have shown previously that a combination treatment using dietary n-3 polyunsaturated fatty acids (n-3 PUFA) in the form of triglycerides and thiazolidinedione (TZD) antidiabetic drugs could efficiently prevent or reverse obesity in mice fed obesogenic high-fat diet, while ameliorating insulin resistance primarily through the effect on skeletal muscle. Recent animal experiments as well as studies in human subjects have also suggested that n-3 PUFA as phospholipids might be superior to triglycerides, especially with regard to the effect on hepatic steatosis. Therefore, in this study, we investigated how n-3 PUFA as phospholipids, either alone or in combination with TZD, affect hepatic steatosis as well as lipid and glucose metabolism.

**Materials and methods:** Adult male C57BL/6N mice were fed for 7 weeks a corn oil-based high-fat diet (cHF; lipids ~35% wt/wt) or various cHF-based treatments: (i) PC, cHF with n-3 PUFA as phosphatidylcholine-rich concentrate replacing ~7 % of dietary lipids; ii) R, cHF with 10 mg rosiglitazone/kg diet; iii) PC+R. Chow-fed mice served as controls for the effect of cHF feeding. Glucose tolerance, markers of glucose and lipid homeostasis, hepatic steatosis, and hepatic gene expression were assessed.

**Results:** Both PC and PC+R but not R prevented weight gain (cHF, 15.9 ± 1.2; PC, 4.9 ± 1.1; R, 12.1 ± 1.9; PC+R, 2.0 ± 0.9 g; p<0.05), while all treatments markedly reduced the weight of abdominal fat depot (cHF, 2.67 ± 0.14; PC, 1.19 ± 0.17; R, 1.86 ± 0.25; PC+R, 0.71 ± 0.12 g; p<0.05). All treatments also reduced plasma levels of triacylglycerols and NEFA, however total cholesterol was lower only in the PC and PC+R groups (cHF, 4.3 ± 0.1; PC, 3.4 ± 0.3; R, 4.0 ± 0.3; PC+R, 3.3 ± 0.2 mmol/l; p<0.05). Both PC and PC+R also improved glucose tolerance (AUC for glucose; cHF, 1.30 ± 0.11; PC, 0.89 ± 0.06; R, 1.09 ± 0.11; PC+R, 0.61 ± 0.13 mol\*180 min; p<0.05). While R tended to increase hepatic lipids, both PC and PC+R treatments completely prevented the development of fatty liver (cHF, 81 ± 3; PC, 40 ± 4; R, 117 ± 25; PC+R, 40 ± 3 mg/g tissue; p<0.05). Furthermore, screening of hepatic gene expression by microarrays showed a complex and significant down-regulation of many biosynthetic pathways including lipogenesis as well as cholesterol and bile acid synthesis in the PC group. Conversely, fatty acid oxidation was stimulated, and the PC+R combination further potentiated this effect. The expression of key regulatory genes within each pathway was verified by quantitative real-time RT-PCR and yielded a perfect conformity with microarrays.

**Conclusion:** Dietary n-3 PUFA as phospholipids efficiently ameliorated obesity-associated metabolic defects such as dyslipidemia and glucose intolerance, while completely preventing the development of fatty liver. The latter effect was associated with a complex inhibition of biosynthetic pathways in the liver. Prevention of weight gain and improvement in glucose tolerance were further potentiated when PC was combined with a low dose of TZD, suggesting its potential use in clinical settings.

*Supported by: Czech Science Foundation (P301/10/1420), EFSD New Horizons grant*

## 643

### Itch, an E3 ubiquitin ligase, is a new target for nonalcoholic steatohepatitis

A. Marino, M. Cardellini, R. Menghini, F. Davato, A. Peschiaroli, P. Sbraccia, P. Gentile, G. Melino, R. Lauro, M. Federici;

Medical School, University Tor Vergata, Rome, Italy.

**Background and aims:** Nonalcoholic steatohepatitis (NASH) is a complication of nonalcoholic fatty liver disease (NAFLD) occurring in 10–20% of patients affected. The molecular mechanisms underlying the transition from NAFLD to NASH are unclear. We performed a microarray analysis to compare human livers from severe obese subjects with normal glucose tolerance



and simple hepatic steatosis (NGT/SS) to patients with severe obesity, diabetes and steatohepatitis (DM2/SH) to identify potential targets involved in the transition from NAFLD to NASH. Among the down regulated genes in DM2/SH we focused on *itch*/AIP4. This gene encode for an E3 ubiquitin ligase whose role is to target different proteins, involved in JNK and NF $\kappa$ B pathway, for proteasomal degradation. *Itch* is also known to be involved in the development of different types of cancers.

**Materials and methods:** Microarrays studies were performed comparing 3 NGT/SS to 3 DM2/SH; results were validated in a cohort of 25 subjects with severe obesity and different degrees of hepatic steatosis and inflammation. To investigate the potential role of *itch* in development of steatohepatitis we used *in vitro* and *in vivo* model of fatty liver disease. Murine hepatocytes cells were cultured in presence or not of FFAs (palmitic acid 0.5mM, oleic acid 1mM) for 6 h; expression of *itch* and its targets was evaluated by real-time PCR and western blot. WT mice fed standard chow and high fat diet (HFD, 60% cal from fat) for 16 weeks were subjected to *itch* liver expression analysis by real-time PCR and western blot. We also studied the expression of *itch* in mice treated with HFD and the hepatic procarcinogen diethylnitrosamine (DEN) as an inducer of hepatocellular carcinoma (HCC).

**Results:** In subjects with DM2/SH we confirmed significantly reduced *itch* expression in liver biopsies by real-time PCR and western blot ( $p<0.05$ ). Immunohistochemical analysis of liver biopsies showed reduced *itch* expression in cytoplasm and nuclei of hepatocytes in steatotic areas. In FFAs stimulated hepatocytes compared with untreated cells, we observed a significant reduced mRNA expression and protein levels of *itch* ( $p<0.01$  and  $p<0.05$ , respectively) and an increase in *itch* targets RIP1 and c-FLIP<sub>L</sub> ( $p<0.05$ ). We questioned if *itch* expression could be reduced in animal model of diet induced obesity and hepatic steatosis. Total liver mRNA and protein analysis showed decreased *itch* expression in liver but not adipose tissue from WT mice fed HFD for 16 weeks compared to control mice fed normal diet (ND) ( $p<0.001$ ). We confirmed that deficit in *itch* expression was predominant in hepatocytes cells by gene expression analysis comparing purified hepatocytes to non parenchymal cells isolated from HFD and ND mice liver tissues. Similarly to *in vitro* studies, levels of *itch* targets were increased ( $p<0.05$ ). In a model of obesity-related HCC we found that *itch* expression was reduced in tumor areas compared to tumor free areas ( $p<0.05$ ). Given the effect of FFA and HFD on *itch* expression and function, we studied whether *itch* KO mice are more sensitive to HFD.

**Conclusion:** Our results suggest that *itch* down regulation occur during steatohepatitis development and can influence the course of disease. Further studies must be performed to understand if *itch* could be considered a predictor of poor prognosis and eventually used as prognostic marker in nonalcoholic fatty liver disease.

## PS 045 Novel players in the development of insulin resistance

644

### Differential effects of antibiotics on intestinal microbiota composition and insulin resistance in obese humans; a randomised controlled trial

A. Vrieze<sup>1</sup>, C. Out<sup>2</sup>, L. Jonker<sup>1</sup>, F. Holleman<sup>1</sup>, G.M. Dallinga-Thie<sup>1</sup>, M.T. Ackermans<sup>3</sup>, M.J. Serlie<sup>3</sup>, F. Knop<sup>4</sup>, J.J. Holst<sup>4</sup>, H.G.H. Heilig<sup>5</sup>, W.M. de Vos<sup>5,6</sup>, A.K. Groen<sup>3</sup>, E.G. Zoetendal<sup>5</sup>, J.B.L. Hoekstra<sup>1</sup>, M. Nieuwdorp<sup>1</sup>;

<sup>1</sup>Department of Internal & Vascular Medicine, Academic Medical Center, Amsterdam, Netherlands, <sup>2</sup>Department of Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, Netherlands, <sup>3</sup>Department of Endocrinology and Metabolism, Academic Medical Center, Amsterdam, Netherlands, <sup>4</sup>Department of Internal Medicine, Gentofte Hospital, Hellerup, Denmark, <sup>5</sup>Laboratory of Microbiology, Wageningen University, Netherlands, <sup>6</sup>Department of Basic Veterinary Medicine, University of Helsinki, Finland.

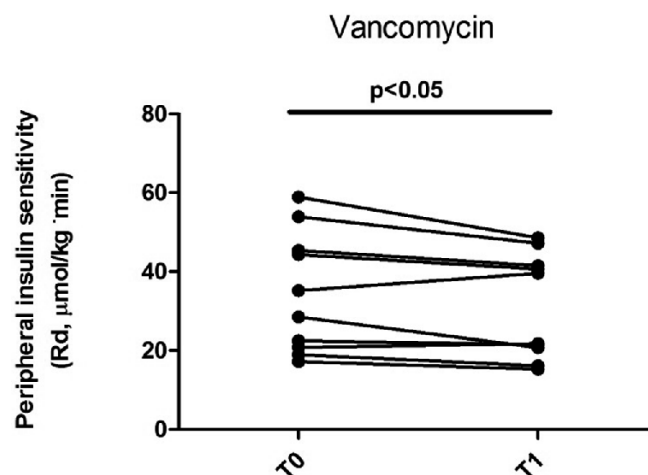
**Background and aims:** Recent data in animal models reveal that obesity is associated with substantial changes in composition and metabolic function of intestinal microbiota. Moreover, intestinal microbiota modulation with antibiotics against gram positive bacteria alters oral glucose tolerance in mice. We hypothesized that short term oral antibiotic treatment affects faecal microbiota composition and subsequent glucose metabolism in obese male subjects with metabolic syndrome.

**Materials and methods:** In this randomised controlled trial, 20 male subjects with metabolic syndrome (no medication use) were randomised to either 7 days amoxicillin 500 mg tid ( $n=10$ ) or 7 days vancomycin 500 mg tid ( $n=10$ ). We studied insulin resistance (by hyperinsulinaemic normoglycaemic clamp with stable isotopes) and intestinal microbiota composition before and after these antibiotics.

**Results:** We found a significant decrease of peripheral insulin sensitivity in the vancomycin treatment group (median Rd from 31.9 to 30.7  $\mu\text{mol/kg/min}$ ,  $p<0.05$ , Figure 1), whereas amoxicillin did not have a significant effect (median Rd from 26.7 to 25.8  $\mu\text{mol/kg/min}$ ,  $p=\text{ns}$ ). There was no change in hepatic insulin sensitivity in any of the groups. An increase in fasting glucagon levels was seen upon vancomycin treatment (median glucagon from 80 to 95 ng/L,  $p<0.01$ ), whereas amoxicillin had no effect (median glucagon from 72 to 67 ng/L,  $p=\text{ns}$ ).

**Conclusion:** Reducing gram positive gut microbiota by vancomycin affects peripheral insulin resistance and glucagon secretion in obese subjects with metabolic syndrome, underscoring the potential role of intestinal microbiota in the regulation of glucose metabolism. Impact of antibiotic treatment on the intestinal microbiota composition will be presented and discussed.

**Figure 1.** Individual change in peripheral insulin sensitivity after vancomycin



Clinical Trial Registration Number: NTR 2566

## 645

**Obesity alters clock gene expression in human visceral adipose tissue**E. Gonzalez-Ruano<sup>1,2</sup>, E. Vieira<sup>1,2</sup>, F.A. Hanzu<sup>3</sup>, A. Jimenez<sup>4</sup>, Y. Esteban<sup>1,2</sup>, R. Gomis<sup>1,2</sup>;<sup>1</sup>CIBERDEM, <sup>2</sup>IDIBAPS, <sup>3</sup>Endocrinology and Nutrition Unit Hospital Clinic, <sup>4</sup>Endocrinology and Nutrition Unit, Hospital Clinic, Barcelona, Spain.

**Background and aims:** Disturbances in the circadian rhythms have emerged as one of the possible causes in the development of obesity and type 2 diabetes. Indeed, changes in the sleep patterns because of night shift works are related with higher risk to develop obesity and type 2 diabetes, highlighting the importance of circadian clock in the metabolic and energetic regulation. The central pacemaker is located in the suprachiasmatic nuclei in the hypothalamus it is controlled by a transcriptional/translational feedback loops involving a set of clock genes. Recently, it has been shown that the human adipose tissue has a fully functional circadian clock gene mechanism whose expression is associated with different components of metabolic syndrome. Thus, the aim of the study was to identify the main clock genes that might be disrupted in visceral adipose tissue from lean and obese subjects.

**Materials and methods:** Visceral adipose tissue was obtained from lean and obese women patients (n=8; age= 47.6± 4.2 in obese vs 46.3±7.2 in lean) that were subjected to bariatric surgeries at our hospital. The day before the patients were synchronized at having lunch and dinner and the AT biopsies were taken between the 11.00 and 13.00. The mRNA expression of clock genes was performed by real time PCR. Total RNA was extracted using RNeasy lipid mini kit (Qiagen). Reverse transcription was performed with random hexamers as primers and Superscript III (Invitrogen). Quantitative real-time PCR was carried out in LightCycler 480 (Roche) using MESAGreen (Eurogentec) and specific primers for each gene analyzed. Expression analysis was done with 2-ΔΔCt method. For statistical analysis paired t-Student test was performed using Graphpad prism 5.0 software

**Results:** The baseline clinical and metabolic characteristics shows differences in insulin resistance estimated by HOMA-IR (5.8 ± 1.8 vs < 2.93 ), plasma insulin (202.8 ± 78.5 vs 15.3-98.6 pmol/L) and adiponectin (12.8 ± 3.5 vs 16.7-23.3 ug/ml ) between obese and lean subjects, found no differences in other clinical variables analyzed. First, we compared the expression levels of main clock genes, Rora, Rev-erba, Bmal1, Cry1, Cry2, Per1 and Per2 with the expression levels of the gene Clock within the lean and the obese group. In the lean group, the mRNA expression of clock genes was very low with the highest expression detected in Per1 mRNA levels. In the obese group, clock and Rev-erbalpha had the same gene expression levels whereas Per1 had a 6 times higher expression levels and Cry2 had 3 times higher expression levels than clock. When we compared lean versus obese subjects, the mRNA levels of Cry2 were increased in obese patients compared to lean subjects as well as there is a strong tendency for the upregulation of Clock in obese patients (p=0.053) versus lean subjects. Interestingly, the nuclear receptor Rev-erba which was suggested to link circadian rhythms and metabolism had 3 times higher expression levels of in obese patients compared to lean subjects.

**Conclusion:** Our results show that obesity alters the expression pattern of Cry2, Clock and Rev-erbalpha in human visceral adipose tissue. The clock gene specific changes in human visceral adipose tissue can help to develop new strategies and therapies for the treatment of obesity.

*Supported by: MICINN, Generalitat de Catalunya*

## 646

**TNF-related apoptosis-inducing ligand significantly attenuates metabolic abnormalities in high fat fed mice reducing adiposity and systemic inflammation**S. Bernardi<sup>1</sup>, G. Zauli<sup>2</sup>, C. Tikellis<sup>3</sup>, C. Bruce<sup>3</sup>, M. Febbraio<sup>3</sup>, R. Candido<sup>4</sup>, B. Fabris<sup>1</sup>, P. Secchiero<sup>2</sup>, M.E. Cooper<sup>3</sup>, M.C. Thomas<sup>3</sup>;<sup>1</sup>University of Trieste, Italy, <sup>2</sup>University of Ferrara, Italy, <sup>3</sup>Baker IDI, Melbourne, Australia, <sup>4</sup>Centro Diabetologico, Trieste, Italy.

**Background and aims:** Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) has recently been shown to ameliorate the natural history of diabetes mellitus (DM). It has not been determined yet whether systemic TRAIL delivery would prevent the metabolic abnormalities due to a high fat diet (HFD).

**Materials and methods:** For this purpose 27 male mice C57bl6 aged 8 weeks were randomly allocated to standard diet, HFD or HFD + TRAIL for 12 weeks. TRAIL was delivered weekly by intra-peritoneal injection. Body composition was evaluated; indirect calorimetry studies, glucose tolerance test

(GTT) and insulin tolerance tests (ITT) were performed. Pro-inflammatory cytokines together with adipose tissue gene expression and apoptosis were measured.

**Results:** TRAIL treatment reduced significantly the increased adiposity associated with a HFD. Moreover, it reduced significantly hyperglycaemia and hyperinsulinemia during a GTT and it improved significantly the peripheral response to insulin. TRAIL reversed the changes in substrate utilization induced by HFD and ameliorated skeletal muscle free fatty acid oxidation rate. This was associated with a significant reduction of pro-inflammatory cytokines together with a modulation of adipose tissue gene expression and apoptosis.

**Conclusion:** This data sheds light on the possibility of fat regulation by TRAIL and opens new therapeutic possibilities against obesity, systemic inflammation and type 2 DM.

## 647

**The role of glucocorticoids in glucose intolerance development in MIF-deficient mice**

I. Stojanovic, I. Nikolic, T. Saksida, M. Vujicic, S. Stosic-Grujicic; Immunology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Serbia.

**Background and aims:** Macrophage migration inhibitory factor (MIF) is a cytokine with versatile functions including promotion of inflammation, regulation of glucose metabolism and inhibition of immunosuppressive effects of glucocorticoids. It has been established that MIF deficiency provokes beta cell dysfunction, development of glucose intolerance and obesity in mice. Since elevated glucocorticoids are implicated in diabetes development, the aim of this study was to explore the relationship between MIF and the level of glucocorticoids in disease development.

**Materials and methods:** MIF-deficient mice (MIF-KO) and their wild type (WT) counterparts C57BL/6 were first tested for the concentration of corticosterone in the serum by ELISA, for the expression of glucocorticoid receptor (GR) in spleen cells and pancreatic lymph node cells by flow cytometry, and for the expression of GR in pancreatic islets by Western blot analysis. Next, mice were treated either with a GR inhibitor mifepristone (20 mg/kg bw) or with sesame oil and DMSO in the same concentration (diluent) for 7 days. Intraperitoneal glucose tolerance test was performed before and after the end of the treatment, while blood glycemia was measured weekly during the next five weeks. Insulin secretion from *in vitro* cultured pancreatic islets was determined by ELISA. Statistical significance between groups was calculated by ANOVA *t* test.

**Results:** Serum corticosterone was elevated in MIF-KO animals compared to WT mice, while the expression of glucocorticoid receptor in lymphoid tissues was similar to the one seen in C57BL/6 mice. Pancreatic islets from MIF-KO seemed to have lower protein expression of GR. After *in vivo* GR inhibition glucose tolerance in MIF-KO mice significantly improved compared to diluent-treated mice. Although blood glucose level in mifepristone-treated MIF-KO mice was lower immediately after the end of the treatment, it rose over time and after 4 weeks returned to the level observed in diluent-treated mice. Mifepristone did not improve already impaired function of beta cells from MIF-KO mice.

**Conclusion:** Elevated glucocorticoids in MIF-KO mice are probably a result of the absence of negative feedback mechanisms mediated by MIF. This excess of glucocorticoids could partly account for the observed development of glucose intolerance in MIF-KO mice since GR inhibition transiently improved control of glycemia. These results give new insight in the delicate balance between MIF and glucocorticoids in diabetes development.

*Supported by: Ministry of Education and Science, Republic of Serbia (No.: 173013).*

## 648

**Deficiency of tetrahydrobiopterin induces insulin resistance and progression of diabetes**

A. Abudukadier<sup>1</sup>, Y. Fujita<sup>1</sup>, A. Obara<sup>1</sup>, A. Ohashi<sup>2</sup>, T. Fukushima<sup>1</sup>, Y. Sato<sup>1</sup>, Y. Nakamura<sup>1</sup>, S. Yamane<sup>1</sup>, M. Ogura<sup>1</sup>, M. Hosokawa<sup>1</sup>, H. Hasegawa<sup>2</sup>, N. Inagaki<sup>1</sup>;

<sup>1</sup>Department of Diabetes and Clinical Nutrition, Graduate School of Medicine, Kyoto University, <sup>2</sup>Department of Functional Morphology, Nihon University School of Medicine, Tokyo, Japan.

**Background and aims:** Endothelial nitric oxide synthase (eNOS) dysfunction occurs in conditions of diabetes and insulin resistance. In addition, eNOS dysfunction is known to be involved in deteriorating insulin sensitivity and glucose metabolism. These findings suggest that the factors which regulate eNOS activity also play an important role in regulating insulin sensitivity and glucose metabolism. Tetrahydrobiopterin (BH4) is an essential co-factor of eNOS, and is synthesized mainly in liver. Our previous study showed that liver BH4 content was decreased in conditions of diabetes and that its supplementation suppressed gluconeogenesis in liver and lowered blood glucose levels eNOS-dependently. The aim of this study was to investigate the effect of BH4 deficiency on progression of diabetes and insulin resistance using the mouse model of BH4 deficiency.

**Materials and methods:** We used hph-1 mice and control mice of the same background (C57BL/6×CBA mice). The hph-1 mouse exhibits a genetic 90% deficiency in GTP-cyclohydrolase I (GTPCH I), a rate-limiting enzyme in BH4 syntheses, and a marked reduction in BH4 levels. Blood glucose levels and plasma insulin levels of these mice were measured. Intraperitoneal glucose tolerance test (IPGTT), insulin tolerance test (ITT), and pyruvate tolerance test (PTT) were performed. Saline with or without 10mg/kg BH4 was administered twice daily intraperitoneally for 7 consecutive days and blood glucose levels were measured. Protein expressions of AMP activated protein kinase (AMPK)  $\alpha$ , phospho-AMPK $\alpha$ , AKT, and phospho-AKT were analyzed by western blotting.

**Results:** Six-week old hph-1 mice fed normal chow exhibited overt diabetes: fasting blood glucose levels (hph-1,  $72.1 \pm 2.8$  mg/dl; control,  $48.1 \pm 2.3$  mg/dl;  $p < 0.001$ ) and fed blood glucose levels (hph-1,  $181.5 \pm 2.6$  mg/dl; control,  $147.3 \pm 7.5$  mg/dl;  $p < 0.001$ ). Body weight of the two groups was not statistically different. The hph-1 mice had higher fasting plasma insulin levels (hph-1,  $284.7 \pm 82.4$ ; control,  $97.7 \pm 12.0$ ;  $p < 0.05$ ) and higher fed plasma insulin levels (hph-1,  $900.2 \pm 89.1$  pg/ml; control,  $604.7 \pm 70.7$  pg/ml;  $p < 0.05$ ). In IPGTT, despite the higher plasma insulin levels compared to those in control mice, hph-1 mice exhibited impaired glucose tolerance (expressed as area under the curve (AUC) from 0 to 120 min, hph-1,  $39.1 \times 10^3$  mg/dl×min; control,  $24.7 \times 10^3$  mg/dl×min;  $p < 0.001$ ). ITT data showed that the hph-1 mice had insulin resistance. PTT data showed that hph-1 mice had higher liver glucose production than control mice, and that activation of AMPK was decreased in liver tissues of hph-1 mice. To assess the correlation between BH4 content and glucose metabolism, hph-1 mice were treated with BH4. BH4 treatment significantly lowered blood glucose levels. AMPK and AKT were activated by administration of BH4 in liver tissues of hph-1 mice.

**Conclusion:** These findings suggest that endogenous BH4 regulates systemic glucose metabolism and that its deficiency can induce insulin resistance and progression of diabetes. BH4 and its rate-limiting enzyme GTPCH I are possible targets for treatment and prevention of diabetes.

## 649

**Response of different mouse strains to a high fat diet: BALB/c mice are resistant to diet-induced obesity and insulin resistance**

M.K. Montgomery, N.J. Hallahan, G.J. Cooney, N. Turner; Diabetes & Obesity Research Program, Garvan Institute of Medical Research, Darlinghurst, Australia.

**Background and aims:** Transgenic and knockout mice are major research models used to investigate the aetiology of obesity and type 2 diabetes, with many proposed therapeutic target proteins identified using this approach. However, genetically manipulated mice are produced on a range of different genetic backgrounds and there is only limited information on the inherent propensity of different mouse strains to develop metabolic disease. To investigate strain-dependent differences in the susceptibility of mice to diet-induced obesity and insulin resistance, we have carried out an extensive comparison of the response to a high fat diet (HFD) of five commonly used mouse strains (DBA/2, 129X1/SvJ, BALB/c, C57BL/6 and FVB/N).

**Materials and methods:** Mice were fed either a standard low-fat laboratory diet (LFD; 8% calories from fat) or a HFD (45% calories primarily from lard) for 8 weeks ( $n=8$  for each strain and diet). At the end of the feeding period, body composition was determined using dual-emission X-ray absorptiometry and glucose tolerance was measured during an intraperitoneal glucose tolerance test. Tissues were collected for subsequent analysis.

**Results:** Body weight was higher in three out of five mouse strains (DBA/2, 129X1/SvJ and FVB/N) in response to a HFD, while whole-body fat mass was significantly increased (+40–90%,  $P < 0.001$ ) by fat-feeding in all strains. Glucose tolerance was significantly impaired by the HFD in all strains except BALB/c, where the HFD animals displayed similar glucose tolerance to LFD counterparts. Consistent with these findings, insulin sensitivity (as indicated by HOMA-IR index) was maintained at the level of LFD controls in fat-fed BALB/c mice, but was significantly reduced in all other mouse strains. Tissue triglyceride (TAG) levels were assessed to determine if differential accumulation of lipid in non-adipose tissue may be associated with the disparate effects of the HFD on insulin action. The DBA/2, 129X1/SvJ, C57BL/6 and FVB/N mice all displayed significant increases in muscle and liver TAG in response to the HFD (+70–380%,  $P < 0.001$ ). Intriguingly, BALB/c mice did not accumulate excess liver triglycerides, despite a similar increase in lipid deposition in skeletal muscle compared to other strains. In the liver, there was minimal variation in markers of mitochondrial oxidative capacity across the strains. Despite the lack of TAG accumulation and the maintenance of insulin sensitivity in response to HFD, BALB/c mice were the only strain that displayed an increase in lipid oxidative damage in liver (both lipid hydroperoxide and TBARS).

**Conclusion:** This detailed mouse strain comparison highlights the importance of genetic background in determining the metabolic response to HFD. In addition, we showed that impaired glucose tolerance correlates with lipid accumulation in the liver, but not in skeletal muscle. Furthermore, our findings suggest that accumulation of neutral triglycerides might prevent the formation of other harmful lipid species that cause lipoxidative damage.

Supported by: NHMRC

## 650

**Susceptibility genes for type 2 diabetes and related phenotypes cluster on mouse chromosome 14**

N. Babaya<sup>1</sup>, S. Noso<sup>1</sup>, Y. Hiromine<sup>1</sup>, H. Ueda<sup>2</sup>, H. Ikegami<sup>1</sup>;

<sup>1</sup>Department of Endocrinology, Metabolism and Diabetes, Kinki University Faculty of Medicine, Osaka-sayama, Japan, <sup>2</sup>Department of Molecular Endocrinology, Osaka University Graduate School of Medicine, Suita, Japan.

**Background and aims:** We mapped a quantitative trait locus on chromosome (Chr) 14 (*Nidd2n*) affecting diabetes-related phenotypes in crosses of NSY mice, an animal model of spontaneous type 2 diabetes, with control C3H mice. Recently, we constructed a consomic strain, C3H-14<sup>NSY</sup>, in which NSY-derived whole Chr14 was introgressed onto control C3H background genes, and clarified that the Chr14 harbor major susceptibility gene(s) for type 2 diabetes. The aim of this study is to localize the region and to clarify the function of susceptibility genes for type 2 diabetes on Chr14.

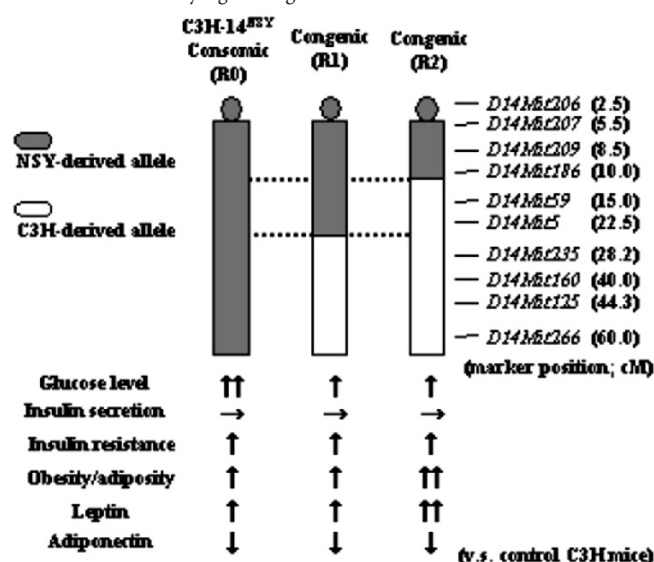
**Materials and methods:** We constructed two novel congenic strains homozygous for different segments of NSY-Chr14 on the control C3H background: R1 strain possesses proximal and middle segment of NSY-Chr14 and R2 strain possesses most proximal segment of NSY-Chr14. Various kinds of diabetes-related phenotypes of congenic strains have been monitored longitudinally in comparison with consomic C3H-14<sup>NSY</sup> and parental NSY and C3H strains.

**Results:** Congenic R1 and R2 mice showed significantly higher blood glucose level than C3H mice ( $p < 0.01$ ). Hyperglycaemia in R1 and R2 mice, however, were not as severe as in consomic C3H-14<sup>NSY</sup> mice ( $p < 0.05$ ). Insulin resistance observed in C3H-14<sup>NSY</sup> mice was retained in both R1 and R2 mice. C3H-14<sup>NSY</sup>, R1 and R2 mice showed severe adiposity than C3H mice ( $p < 0.01$ ). R2 showed significantly higher body weight than C3H mice ( $p < 0.001$ ), in contrast to no significant increase in body weight in R1 and C3H-14<sup>NSY</sup> mice. Leptin level was significantly higher in R1, R2 and C3H-14<sup>NSY</sup> as well as in NSY mice than in C3H mice ( $p < 0.01$  for all). No significant difference was observed in leptin level between R1 and C3H-14<sup>NSY</sup> mice. Leptin level in R2 mice, however, was significantly higher than in C3H-14<sup>NSY</sup> mice ( $p < 0.001$ ), and similar to NSY mice, despite the heavier body weight and fat-pad weight in NSY mice. Serum leptin levels were significantly correlated with abdominal fat pads in NSY ( $r=0.75$ ,  $p < 0.05$ ) and congenic, consomic and parental C3H mice ( $r=0.78$ ,  $p < 0.001$ ). Serum adiponectin level was significantly higher in NSY than in C3H mice ( $p < 0.05$ ), but was significantly lower in C3H-14<sup>NSY</sup>, R1 and R2 than



in C3H mice ( $p < 0.001$ ). Serum adiponectin levels were significantly correlated with abdominal fat pads in congenic, consomic and parental C3H mice ( $r = -0.48$ ,  $p < 0.01$ ), but not in NSY mice.

**Conclusion:** These data provide direct evidence that proximal region of mouse Chr14 harbor a gene or genes for insulin resistance and adiposity/obesity, which interact epistatically with the non-proximal region of Chr14. Serum levels of adiponectin, but not leptin, were dysregulated in NSY mice, which is controlled by a gene or genes outside of Chr 14.



## 651

### Insulin-resistance and low-grade inflammation: the role of low and chronic doses of bisphenol-a on adipocyte function

R. Valentino<sup>1</sup>, V. D'Esposito<sup>2</sup>, F. Passaretti<sup>2,3</sup>, D. Liguoro<sup>1</sup>, A. Rainone<sup>2</sup>, P. Formisano<sup>2</sup>, F. Beguinot<sup>2</sup>;

<sup>1</sup>IEOS-CNR, Naples, <sup>2</sup>DBPCM, University of Naples "Federico II", Naples, Italy, <sup>3</sup>Department of Pharmaceutical Science, University of Salerno, Italy.

**Background and aims:** Bisphenol-A (BPA) is a lipophilic compound, main component of plastic and one of the highest-volume chemicals in commerce. At very low doses displays strong estrogen-like activities, altering several metabolic, but also inflammatory functions. It is detectable at nanomolar levels in human serum and urine worldwide and is detected in the packaging of food, in beverage containers and in material commonly used in dentistry. Considering the BPA bio-accumulation in adipose tissue, the involvement of this compound in obesity, insulin resistance, metabolic syndrome and type II diabetes, all challenging and growing health problems, is suggestive. The aim of the current work is to investigate the effect of low and chronic BPA doses on adipocyte function, in term of insulin signaling and adipokine production. **Materials and methods:** Preadipocytes (fibro3T3-L1) and differentiated (adipo3T3-L1) cells have been cultured for 24-48 and 72 h in presence of 1 nM BPA, and acutely stimulated (10 min) with 100 nM insulin. Fibro3T3-L1 cells have been also cultured continuously in presence of 1 nM BPA for two weeks and during adipogenesis (further ten days). AKT, ERK, STAT and JNK activation pathways were analyzed by Western blot with phospho-specific antibodies. Moreover, different adipokines were assayed in conditioned media using BIOPLEX multipanel assay.

**Results:** After 24-48 and 72h incubation with 1 nM BPA, insulin-stimulated AKT and ERK phosphorylation was down-regulated with no change of protein abundance. These differences were more marked when fibro3T3-L1 and adipo3T3-L1 were incubated chronically with 1 nM BPA. Interestingly, the IL-6 and MIP-1α levels in conditioned media from adipo3T3-L1 were higher in presence of BPA. Moreover, STAT and JNK phosphorylation was also increased, likely as the results of stimulation with inflammatory cytokines.

**Conclusion:** Chronic BPA bio-accumulation in adipose tissue, at very low concentrations, interferes with insulin signaling and adipose cell activity, with possible development of low-grade inflammation. BPA disrupts important cellular functions in adipocytes, such as AKT and ERK activities, leading to insulin resistance. Our hypothesis is that BPA, possibly via estrogen and/or Toll-like receptors, is able to activate pathways leading to low grade inflam-

mation and insulin-resistance, altering the main adipose tissue functions. Further experiments are required to verify the interference of BPA low and chronic doses on adipogenesis and the impact of BPA on insulin action in human insulin-dependent tissues.

## 652

### Palmitate-induced transitory activation of mTORC2/AKT pathway leads to ER stress and cell death independently of mTORC1

T.M. de Araújo<sup>1</sup>, J.A. Faria<sup>1</sup>, A.P. Kinote<sup>1</sup>, A.L. Barbosa<sup>1</sup>, L.A. Velloso<sup>2</sup>, G.F. Anhe<sup>1</sup>;

<sup>1</sup>Pharmacology, <sup>2</sup>Internal Medicine, University of Campinas, Brazil.

**Background and aims:** AKT is a serine/threonine protein kinase that is essential for regulating cell growth, proliferation, survival and the interaction with environmental stimuli. Classically, AKT is fully activated by phosphoinositide-dependent kinase 1 (PDK1) and mammalian Target of Rapamycin (mTOR) complex 2, leading to inhibition of apoptosis. Alternatively, transitory AKT activation was demonstrated to cause cell death through yet not described mechanisms. Excess of free-fatty acids (FFA) are known to contribute to steatosis and cell death in hepatocytes due to activation of ER stress-related pathways. ER stress triggers the Unfolded Protein Response (UPR) that is comprised by three independent branches; the PKR-like ER kinase (PERK), the activating transcription factor (ATF)-6 and serine/threonine-protein kinase/endoribonuclease Ire1. The aim of this study is to demonstrate if short-term AKT activation by palmitate is linked to ER stress and apoptosis in HepG2 cells.

**Materials and methods:** HepG2 cells were treated with palmitate (0,5 mmol/l) pre-conjugated with albumin, rapamycin (500 nmol/l) and/or wortmannin (1 nmol/l). Cells were also transfected with siRNA targeted to Raptor or a siRNA targeted to Rictor. A scrambled siRNA was used as control. mTORC2/AKT pathway and UPR were assessed by Western blotting. Cell death was assessed by DNA fragmentation.

**Results:** Palmitate-induced apoptosis was maximal after 12h of treatment. CHOP, a marker of ER-stress-dependent apoptosis was also modulated by palmitate and peaked 12h after beginning of treatment but was already enhanced as early as 6h after exposition to palmitate. Processed ATF6 was up-regulated 3h after palmitate exposition and these levels were sustained for 12h after treatment with FFA. Palmitate also activated PERK and increased ATF4 expression (respectively after 6 and 12h of treatment). By inhibiting mTORC1/2 with rapamycin we observed a suppression of palmitate-induced UPR activation and apoptosis. In contrast, Raptor knockdown had no effects over palmitate-induced apoptosis. Rictor knockdown, instead, reduced palmitate-induced apoptosis. Similarly, pre-treatment of HepG2 cells with wortmannin reduced palmitate-induced apoptosis.

**Conclusion:** Our results demonstrated that palmitate induces a short-term and transitory AKT activation that leads to long term activation of UPR and apoptosis. This response is likely to be triggered by mTORC2 activation rather than mTORC1.

Supported by: Fapesp

## 653

### Effects of hyperglycaemia on the regulation and expression of the ApoE, Igfbp1 and Foxo1 genes in HepG2 cells

F.G. Ghiraldini<sup>1</sup>, N. Gilbert<sup>2</sup>, M.L.S. Mello<sup>1</sup>;

<sup>1</sup>Structural and Physiological Biology, UNICAMP, Campinas, Brazil,

<sup>2</sup>Edinburgh Cancer Research Center, Edinburgh, UK.

**Background and aims:** Hyperglycemia caused by diabetes mellitus (DM) is known to affect chromatin organization in hepatocytes of non-obese diabetic (NOD) mouse strain. Sirt1, a NAD<sup>+</sup>-dependent deacetylase, has been related to cellular metabolic changes, acting as a nutritional sensor. Together with the transcription factors PGC-1α and Foxo1, Sirt1 could change the expression profiles of genes involved in cell adaptation and response to hyperglycemic conditions. The aim of this study was to establish possible changes in the expression of the genes *ApoE*, *Igfbp1* and *Foxo1* and whether they are associated with an epigenetic signature involving sirtuins, in hepatoma HepG2 cells under hyperglycemic conditions.

**Materials and methods:** HepG2 cells cultivated in an insulin-containing normoglycemic medium (NM) were used as a control (Group 1). Group 2 consisted in cells treated for 48 h with a high glucose medium (HGM) in absence of insulin, in order to simulate a type I DM condition. Group 3 con-

sisted of cells treated as cells of group 2 but that were subsequently returned to NM for 24 h. Cells treated with NM and HGM containing nicotinamide (NIC), a sirtuin inhibitor, for 6 h, were groups 4 and 5, respectively. qPCR was used to evaluate *Apoe*, *Igfbp1* and *Foxo1* gene expressions. The abundance of the H3K9me2 and H3K9Ac markers was analyzed in the promoters of these genes by ChIP.

**Results:** An increase in gene expression was observed in cells of the group 2 in comparison to those of group 1 (*Apoe*:  $3.6 \pm 0.3$ , *Igfbp1*:  $5.3 \pm 0.9$  and *Foxo1*:  $1.5 \pm 0.2$  fold). These expressions returned to basal levels in cells of group 3 (*Apoe*:  $1.6 \pm 0.1$ , *Igfbp1*:  $1.0 \pm 0.1$ ; *Foxo1*:  $0.8 \pm 0.1$  fold). Treatment with NIC in NM (group 4) elicited a different expression response for each gene: while *Apoe* was not affected, *Igfbp1* presented an increase, similarly to group 2 ( $4.7 \pm 0.8$  fold), and *Foxo1* presented a decrease ( $0.6 \pm 0.1$  fold). In cells of group 5 gene expression was similar only to that of group 2. When epigenetic marks on the promoters of these genes were analyzed in cells of groups 2 and 3, a progressive loss of H3K9me2 and H3K9Ac was detected (Table). Whereas a general increase in both markers was found in cells of group 4, no significant change was detected in cells of group 5 (Table).

**Conclusion:** Changes in gene expression in the studied genes, caused by hyperglycemia, showed to be rapidly modulated according to the cell medium composition. Sirtuin activity seemed to be inhibited by HGM, because treatment with NIC showed results similar to those obtained with treatment with HGM only. However, both H3K9Ac and H3K9me2 epigenetic markers presented the same and continuous response. This could indicate either a slower response to changes in medium conditions or a different epigenetic signature of these markers. Difference in sirtuin response apparently occurred only under NM conditions, which could indicate an inactivity of those proteins under HGM conditions in the absence of insulin.

Epigenetic markers on genes promoters in HepG2 cells expressed as % of input						
Genes	Tests	Group 1	Group 2	Group 3	Group 4	Group 5
<i>Apoe</i>	H3K9ac	$3.98 \pm 0.95$	$1.36^* \pm 0.44$	$0.14^{\dagger} \pm 0.03$	$17.29^* \pm 4.69$	$4.56^{\dagger} \pm 0.53$
	H3K9me2	$12.64 \pm 3.21$	$4.80^* \pm 1.18$	$1.08^* \pm 0.29$	$39.10^* \pm 6.59$	$7.57 \pm 1.78$
<i>Igfbp1</i>	H3K9ac	$6.61 \pm 2.03$	$1.71 \pm 0.40$	$0.001^* \pm 0.0004$	$12.66^* \pm 0.63$	$3.03 \pm 0.74$
	H3K9me2	$15.43 \pm 4.11$	$6.27 \pm 1.43$	$1.07^* \pm 0.17$	$16.55^{\dagger} \pm 2.38$	$12.5 \pm 3.72$
<i>Foxo1</i>	H3K9ac	$23.01 \pm 8.27$	$4.63^* \pm 1.85$	$0.24^{\dagger} \pm 0.03$	$15.18^{\dagger} \pm 3.37$	$6.20 \pm 1.66$
	H3K9me2	$25.28 \pm 6.85$	$17.68 \pm 4.76$	$2.13^* \pm 0.36$	$62.99^* \pm 7.55$	$19.52 \pm 4.17$

\*, differences from the Group 1 and  $\dagger$ , differences from the Group 2 at  $P < 0.05$  (T-student). Arithmetic mean  $\pm$  standard error

Supported by: FAPESP (grants n° 08/58067-5 and 2010/50015-6), CNPq (grant no. 301943/2009)

## PS 046 Immune cells and inflammation in type 2 diabetes

654

### Sex steroid-induced changes in circulating monocyte chemoattractant protein-1 levels may contribute to metabolic dysfunction in obese men

D.M. Ouwens<sup>1</sup>, M. Bekaert<sup>2</sup>, B. Lapauw<sup>2</sup>, S. Lehr<sup>1</sup>, S. Hartwig<sup>1</sup>, D. Herzfeld de Wiza<sup>1</sup>, M. Schiller<sup>1</sup>, W. Passlack<sup>1</sup>, Y. Van Nieuwenhove<sup>2</sup>, Y.E. Taes<sup>2</sup>, H. Sell<sup>1</sup>, J. Eckel<sup>1</sup>, J.M. Kaufman<sup>2</sup>, J.B. Ruige<sup>1</sup>;

<sup>1</sup>Biochemistry, German Diabetes Center, Duesseldorf, Germany,

<sup>2</sup>Endocrinology, Ghent University Hospital, Ghent, Belgium.

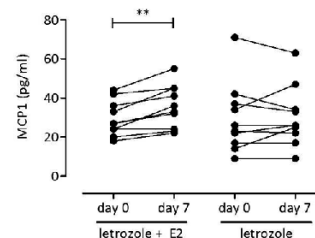
**Background and aims:** Low testosterone accompanied by elevated estradiol associates with the development of metabolic dysfunction in men. Here we explored the hypothesis that alterations in sex steroid levels induce metabolic dysfunction through adipokines.

**Materials and methods:** Circulating levels of sex steroids were quantified by LC-MS/MS and levels of 28 adipokines were determined using magnetic-bead based assays and ELISA in a cross-sectional study of morbidly obese men (n=37, of which 23 had type 2 diabetes (DM2)) and aged-matched controls (n=20), as well as in a randomized clinical trial with healthy young men (n=20) in which obesity-related alterations in sex steroid levels were mimicked by treatment with an aromatase inhibitor plus estradiol patches.

**Results:** Testosterone levels were lowest in morbidly obese men with DM2, intermediate in obese men without DM2, and highest in control men (all  $P < 0.01$ ). Estradiol levels were increased in obese men without DM2 as compared to control men or obese men with DM2 ( $P < 0.05$ ). Adiponectin and omentin levels were lower in obese men, while dipeptidyl peptidase 4, interleukins (IL) IL1Ra, IL5, IL8, IL10, IP10, leptin, and plasminogen-activated inhibitor 1 levels were elevated versus control men (all  $P < 0.05$ ). Adipokine levels were not different between obese men with and without DM2. Testosterone associated negatively and estradiol positively with circulating levels of multiple pro-inflammatory cytokines, including IL1Ra, IL5, IL6, IL10, leptin, monocyte chemoattractant protein 1 (MCP1), and vascular endothelial growth factor (all  $P < 0.05$ ). The associations with estradiol, but not with testosterone, remained significant after adjusting for adipocyte cell size. In a separate clinical trial in healthy young males, circulating levels of MCP1 increased following an intervention in which testosterone was lowered and estradiol levels were raised (figure 1).

**Conclusion:** Alterations in sex steroid levels may contribute to metabolic dysfunction through adverse effects on adipokine levels in obese men. The direct adverse effects on MCP1, a chemokine highly linked to the development of metabolic dysfunction, were substantiated *in vivo* in a trial mimicking obesity-related alterations of sex steroid levels in healthy young males.

**Figure 1.** Changes in MCP1-levels before (day 0) and after (day 7) intervention with an aromatase inhibitor (letrozole) and estradiol patches to lower testosterone and raise estradiol (group letrozole + E<sub>2</sub>), versus intervention with an aromatase inhibitor only to raise testosterone and lower estradiol (group letrozole) in healthy young men. P-values were calculated using a paired Student t-test; \*\*,  $P < 0.01$ .



Clinical Trial Registration Number: NCT00740194

Supported by: Deutsche Diabetes Stiftung

655

### Anti-HSP70 levels and the metabolic syndrome: the Casale Monferrato study

B. Lorenzati, S. Giunti, F. Barutta, S. Pinach, P. Cavallo-Perin, G. Bruno, G. Gruden;

Department of Internal Medicine, University of Turin, Italy.

**Background and aims:** The metabolic syndrome (MS), a cluster of metabolic abnormalities characterized by a low-grade inflammation and a pro-oxidant state, is associated with increased risk of developing both type 2 diabetes (T2DM) and cardiovascular disease. Heat shock protein 70 (HSP70) is an intracellular, highly conserved polypeptide important for cell survival. In stress

conditions, HSP70 can be exposed on the plasma membrane and/or released into the circulation, eliciting an immune response. A reduction in HSPs has been proposed to contribute to impaired insulin signaling/responsiveness characteristic of T2DM. Our aim was to investigate the potential association between circulating anti-HSP70 and MS.

**Materials and methods:** A nested case-control study was performed within the Casale Monferrato Study (CM). The CM study recruited 3,700 non-diabetic subjects, aged 45–74 years, randomly identified through the files of the resident Casale Monferrato population. The present analysis included subjects ( $n=1552$ ) with plasma creatinine levels  $< 2$  mg/dl, CRP levels  $< 3$  mg/dl, and without cardiovascular disease. Among them, we selected as cases ( $n=251$ ) those who had MS (NCEP-ATPIII) and as controls ( $n=170$ ) those without any component of the MS. Stored plasma samples available for the analyses were 180 for cases and 136 for controls. The sample size provides a power of 82% ( $\alpha=0.05$ ) to detect a difference in log-anti-HSP70 within the cohort of at least one-third of standard deviation. Serum levels of anti-HSP70 were measured by a commercial ELISA kit (Stressgen).

**Results:** Anti-HSP70 levels were higher in subjects with MS than in those without MS, even after adjustment for age and sex (118.2 vs. 106.1  $\mu\text{g/ml}$ ,  $p=0.02$ ). In logistic regression analyses, higher levels of log-anti-HSP70 conferred higher ORs for MS and this remained statistically significant after adjustment for age and sex [OR = 2.02 (1.12–3.66)]. In this model, trend of ORs across quartiles of anti-HSP70 was statistically significant ( $p=0.04$ ). Subjects with anti-HSP70  $> 108.0$   $\mu\text{g/ml}$  had 77% increased odds of having MS compared to those with levels  $\leq 108.0$   $\mu\text{g/ml}$ .

**Conclusion:** In this cohort of non-diabetic subjects at low cardiovascular risk, we found an independent association between anti-HSP70 and MS. This suggests that anti-HSP70 may be a novel marker of MS.

*Supported by: Progetto Ricerca Sanitaria Finalizzata Regione Piemonte*

## 656

### Blood monocytes in type 2 diabetes have an altered microRNA profile

L. Baldeon Rojas<sup>1</sup>, V. Rockova<sup>2</sup>, K. Derks<sup>3</sup>, A. van Oudenaren<sup>1</sup>,

H.A. Drexhage<sup>1</sup>, A.J. van Zonneveld<sup>4</sup>, P.J.M. Leenen<sup>1</sup>;

<sup>1</sup>Immunology, Erasmus MC, Rotterdam, Netherlands, <sup>2</sup>Biostatistics and Hematology, Erasmus MC, Rotterdam, <sup>3</sup>Genetics, Erasmus MC, Rotterdam, <sup>4</sup>Nephrology, Leiden University Medical Center, Netherlands.

**Background and aims:** Type 2 diabetes is associated with low-grade systemic inflammation. This is reflected, amongst others, in vascular complications caused by increased endothelial damage and decreased ability to repair. Bone marrow-derived endothelial progenitor cells (EPC) play an essential role in repairing vascular damage and these cells are functionally inhibited in diabetes. Myeloid EPC are closely related to monocytes/macrophages and also these cells show increased pro-inflammatory activity in type 2 diabetes. To understand the alterations of monocytes at the molecular level and to determine the putative role of microRNAs, we determined microRNA profiles of monocytes from individuals with type 2 diabetes.

**Materials and methods:** From 34 type 2 diabetic patients and 25 age- and sex-matched controls, from two cohorts (German and Ecuadorian), CD-14positive blood monocytes were isolated using Ficoll and MACS separation. Total RNA was used to perform microRNA profiling in an LNA-based array. Differential expression and prediction signature were assessed using correction for multiple testing.

**Results:** Of 711 microRNAs tested, we found 142 microRNAs to be differentially expressed by CD14<sup>+</sup> monocytes from patients with type 2 diabetes compared to matched controls. Furthermore, a set of 15 microRNAs could serve as optimal predictor of disease. Cluster analysis on the basis of the expression of these microRNAs gave a high degree of distinction between patient samples and controls. Using Ingenuity pathway analysis, we corroborated that mainly inflammatory networks involving TNF, NF- $\kappa$ B and its regulatory molecules (SOCS) are potential targets of these microRNAs.

**Conclusion:** Our current findings indicate that the microRNA profile of monocytes in type 2 diabetes is significantly altered. Thus, microRNAs may play an important role in the inflammatory dysregulation of the cells and their functional incompetence in maintaining endothelial integrity.

*Supported by: Dutch Diabetes Research Foundation*

## 657

### Glucose metabolism disorders in kidney transplant recipients associate with higher level of interleukin 1 receptor

A. Witkowska<sup>1</sup>, A. Strozik<sup>1</sup>, S. Gorczyńska - Kosiorz<sup>1</sup>, W. Trautsohl<sup>1</sup>, A. Owczarek<sup>2</sup>, G. Wystrychowski<sup>1</sup>, K. Nabrdalik<sup>1</sup>, W. Grzeszczak<sup>1</sup>, J. Gumprecht<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, Diabetology and Nephrology, Medical University of Silesia, Zabrze, <sup>2</sup>Division of Statistics, Medical University of Silesia, Sosnowiec, Poland.

**Background and aim:** Diabetes is a chronic inflammatory disease promoted by alteration of function of immune system. Renal transplant recipients are at risk of diabetes depending on interplay of immune system disorders characteristic of chronic kidney disease, and modulations of systemic pro- and anti-inflammatory cytokines expression by immunosuppressive therapies. This study aimed to assess whether glucose metabolism disorders observed in patients after renal transplantation are associated with changes in serum level of anti-inflammatory interleukin 10 (IL-10), pro-inflammatory interleukin 1- $\beta$  (IL-1 $\beta$ ) or its receptor (IL-1R).

**Materials and methods:** Seventy-seven patients (45 men and 32 women, aged  $47.6 \pm 11.1$  years) after kidney transplantation, not affected with acute or chronic concomitant inflammatory diseases (potentially influencing serum cytokine level), receiving standard schemes of immunosuppressive therapy, were studied. Upon the results of the oral glucose tolerance test, 43 patients with glucose metabolism disorder (impaired fasting glucose/impaired glucose tolerance or diabetes) and 34 with euglycemia (fasting serum glucose  $< 100$  mg/dl) were identified. The serum levels of IL-1 $\beta$ , IL-1R and IL-10 were measured using ELISA test and compared between the two studied groups with adjustment for age, sex, BMI, serum creatinine, CRP level and lipid profile. T-student test (in case of normal distribution or after normalisation) was applied to discern factors differentiating the patients with normoglycemia or glucose metabolism disorder. Distribution of variables was evaluated by the Shapiro-Wilk test. Homogeneity of variances was assessed by the Levene test.

**Results:** Serum IL-1R level differed statistically significantly between the analyzed groups with higher values in patients with glucose metabolism disorders (Me: 733.4 vs. 429.1;  $p < 0.001$ ). The prediabetic or diabetic patients were also older ( $50.0 \pm 11.6$  vs.  $44.5 \pm 10.0$ ;  $p < 0.05$ ), had higher BMI values ( $28.0 \pm 4.7$  vs.  $25.7 \pm 2.3$ ;  $p < 0.05$ ) and lower HDL level ( $1.27 \pm 0.30$  vs.  $1.57 \pm 0.66$ ;  $p < 0.05$ ). There were no differences in serum levels of CRP, uric acid, creatinine, total and LDL cholesterol and triglycerides. The studied serum level of IL-1 $\beta$  and IL-10 did not differ statistically significantly between the analyzed groups, either.

**Conclusion:** Glucose metabolism disorders in transplant recipients are associated with a rise in IL-1R serum level. This suggests potential role of IL-1R as a marker or the therapeutic goal in these patients, however, larger studies should yield verification of this finding.

## 658

### Targeting the association of calgranulin B (S100A9) with insulin resistance and type 2 diabetes

J.M. Mercader<sup>1</sup>, E.J. Ortega<sup>2</sup>, J.M. Moreno-Navarrete<sup>2</sup>, M. Sabater<sup>2</sup>, N. Pueyo<sup>2</sup>, G. Pardo<sup>2</sup>, M. Serrano<sup>2</sup>, W. Ricart<sup>2</sup>, E. Delgado<sup>3</sup>, R. Burcelin<sup>4</sup>, G. Frühbeck<sup>5</sup>, F. Bosch<sup>6</sup>, G. Mingrone<sup>7</sup>, A. Zorzano<sup>6</sup>, J.M. Fernandez-Real<sup>2</sup>;

<sup>1</sup>Joint IRB-BSC program on Computational Biology, Barcelona, Spain, <sup>2</sup>Department of Diabetes, Endocrinology and Nutrition (UDEN), Institut d'Investigació Biomèdica de Girona (IdIBGi), Girona, Spain, <sup>3</sup>Hospital Central de Asturias, Oviedo, Spain, <sup>4</sup>INSERM Unité 858, Institut de Médecine Moléculaire de Rangueil, Toulouse, France, <sup>5</sup>Clínica Universitaria de Navarra, Pamplona, Spain, <sup>6</sup>Departament de Bioquímica i Biologia Molecular, Institute for Research in Biomedicine (IRB Barcelona), Spain, <sup>7</sup>Institute of Internal Medicine, Rome, Italy.

**Background:** Extensive efforts are being made trying to identify genes involved in the development of type 2 diabetes (T2D). *Calgranulin B* (S100A9) was recognized as a candidate T2D gene in a platform based on the genomic profiling of muscle from a rodent model of T2D, identifying the human orthologs of genes localized in T2D susceptibility regions.

**Methods:** Circulating and S100A9 expressions in human muscle and adipose tissue, isolated fat cells, and in mice models were evaluated. A common 5'-upstream SNP (rs3014866) for S100A9 was studied ( $n=1,450$ ). The effects of weight loss on circulating and S100A9 mRNA in adipose tissue were also investigated. Treatments *in vitro* with recombinant S100A9 were performed.



**Results:** *S100a9* expression was increased in the muscle of diabetic mice, and further confirmed in human muscle from insulin resistant subjects. The rs3014866 SNP was found to be significantly associated with circulating S100A9, and the risk of T2D (TT carriers having increased S100A9 and 28% lower risk). Indeed, increased circulating S100A9 and adipose tissue expression in TT carriers run in parallel to decreased fasting glucose and glycated hemoglobin. In agreement with these results, metformin led to increased S100A9 expression in *ex vivo* treated human adipose tissue explants. On the other hand, obese subjects showed a compensatory increase in circulating S100A9 and adipose tissue expression, as demonstrated by decreased levels after weight loss. While lipopolysaccharide led to increased *S100a9* mRNA in fat from mice, recombinant S100A9 down-regulated inflammation in adipocytes.

**Conclusion:** Current findings support the strategy of testing differentially expressed genes in mice, and their human orthologs in hot genetic regions for T2D. The increased S100A9 levels reported for obesity and insulin resistance may be envisioned as a compensatory mechanism for inflammation.

**Supported by:** MEC (GEN2001-4758, SAF2008-02073), ISCIII (ISCIII RETIC RD06, REDIMET)

## 659

### B- and T-cell deficiency does not protect from obesity-induced insulin resistance and inflammation

D.B. Ballak, R. Stienstra, A. Hijmans, L.A.B. Joosten, M.G. Netea, C.J. Tack; General Internal Medicine, Radboud University Medical Centre, Nijmegen, Netherlands.

**Background and aims:** Obesity is associated with development of chronic low grade inflammation that arises from adipose tissue. Obesity-induced inflammation is associated with insulin resistance and morphologically characterized by macrophage influx into the adipose tissue. More recently, various other immune cells including B- and T-cells have been shown to participate in modulating adipose tissue inflammation during the development of obesity. Here we tested the effect of the absence of both B- and T-cells in mice on the development of obesity-induced insulin resistance and inflammation. **Material and methods:** Wild-type and *Prkdc<sup>-/-</sup>* SCID animals, lacking both B- and T-cells, were given a low-fat (LFD) or high-fat (HFD) diet for 8 weeks to induce obesity.

**Results:** FACS analysis revealed that HFD-feeding modulated the influx of B and T-cells into adipose tissue of obese animals. After 8 weeks of high fat diet feeding, SCID mice displayed a lower bodyweight paralleled by lower circulating leptin levels, a reduction in adipose tissue mass and smaller adipocytes compared to wild-type mice. Circulation levels of insulin were increased due to the HFD feeding in both genotypes, although absolute values were significantly lower in SCID animals. Despite a lower bodyweight after HFD-feeding, gene expression levels of CD68, F4/80, MCP-1 and TNF- $\alpha$  in white adipose tissue were higher, indicating that SCID mice were not protected against HFD-induced inflammation. Moreover, the inflammatory status of the adipose tissue appeared to be more pronounced as stimulation of bone-marrow derived macrophages with conditioned medium from adipose tissue explants of HFD-fed SCID mice led to increased IL-6 and KC levels compared to medium obtained from adipose tissue of HFD-fed wild-type animals. Additionally, systemic KC levels were increased in SCID-mice as compared to wild-type animals. In line with these results, glucose tolerance was impaired in SCID mice after HFD feeding.

**Conclusion:** B- and T-cell deletion does not protect against HFD-induced insulin resistance and inflammation. In contrast, SCID mice showed an increased pro-inflammatory status at the level of the adipose tissue. Our findings suggest that other immune cell populations drive the macrophage accumulation in adipose tissue.

## 660

### High-fructose corn syrup (HFCS) diet induces an NLRP-3 inflammasome-mediated response in the kidney: protection by PPAR $\beta/\delta$ agonist

E. Benetti, M. Collino, M. Rogazzo, M. Aragno, R. Mastrocola, R. Fantozzi; University of Turin, Italy.

**Background and aims:** Fructose-based sweeteners are the most commonly added sugars and High-Fructose Corn Syrup (HFCS: 55% fructose, 42% glucose and 3% higher saccharides) is the less expensive and most widely

used. Consumption of sugar-enriched foods and beverages has been associated with increased prevalence of obesity, insulin resistance, dyslipidemia and cardiovascular injury. However, the molecular mechanism(s) underlying sugar detrimental effects are not completely understood. We have recently demonstrated, in rats, that chronic exposure to a fructose- and cholesterol-enriched diet caused not only metabolic damage but also hepatic inflammation, decreased by pioglitazone, a selective agonist of the Peroxisome Proliferator Activated Receptor (PPAR) $\gamma$ . Here we investigated the deleterious effects of a HFCS-enriched diet, focusing on the renal toxicity of fructose, whose main metabolite is uric acid. As NLRP-3 (NOD-like receptor pyrin domain-containing 3) inflammasome is a recently discovered signaling pathway involved in insulin-resistance and obesity-induced inflammation, we have also studied whether it may contribute to the HFCS-induced pathological effects. We also evaluated whether selective activation of the less investigated PPAR isoform, PPAR $\beta/\delta$ , exerted protective effects.

**Materials and methods:** Male C57BL/6 mice (n=10 per group) were provided with a standard diet or a standard diet plus 15% HFCS for 7 months. Subset of animals were treated with the selective PPAR $\beta/\delta$  agonist GW0742 (1 mg/kg-1, p.o.) for the last 3 months. Body weight, drink and food intake, blood pressure and urine volume were recorded monthly. At the end of the treatment, glucose tolerance test was performed. The expression of PPAR $\beta/\delta$ , fructokinase, NLRP-3 and caspase-1 activation were assessed by western blot on liver and kidney specimens. Serum levels of uric acid and interleukin (IL)-1 $\beta$  were evaluated by ELISA. Data were assessed by one-way ANOVA followed by Bonferroni's post hoc test and a P value <0,05 was considered significant.

**Key results:** In comparison to standard diet, HFCS diet impaired glucose tolerance and induced hyperlipidemia (total cholesterol 3,86 $\pm$ 0,19 mmol/l versus 7,46 $\pm$ 0,25 mmol/L), hyperuricemia (3 $\pm$ 0,05 mg/dl vs 3,9 $\pm$ 0,1 mg/dl) and albumin/creatinine ratio increase (105 $\pm$ 3,3  $\mu$ g/mg vs 148 $\pm$ 1,6  $\mu$ g/mg). Notably, GW0742 evoked a significant improvement in lipid metabolism, insulin responsiveness, hyperuricemia and albuminuria. Interestingly, while HFCS diet evoked a two-fold increase in hepatic fructokinase and renal NLRP-3 expression, chronic GW0742 administration reported the protein levels back to control values. GW0742 ability to affect HFCS-induced NLRP-3 activation was confirmed by a significant reduction in renal caspase-1 processing. Consistently, the increase in serum IL-1 $\beta$  concentration due to HFCS exposure (105,7 $\pm$ 3,33 vs 76 $\pm$ 4,76 mg/dl) was reduced by 60% in the GW0742 group.

**Conclusion:** Our data clearly demonstrate that PPAR $\beta/\delta$  activation improves metabolic damage and reduces renal inflammation due to chronic exposure to the HFCS diet. Reduction in hepatic fructokinase and renal NLRP-3 inflammasome activity significantly contributes to the protective role of GW0742 against deleterious effects of high fructose exposure.

## 661

### TNF-alpha up-regulates TNF-alpha converting enzyme (TACE) in visceral adipose tissues via JNK and PKC activation

H. Motoshima, S. Kawasaki, T. Kondo, S. Hanatani, Y. Takaki, M. Igata, T. Matsumura, T. Senokuchi, T. Nishikawa, E. Araki; Metabolic Medicine, Kumamoto University, Japan.

**Background and aims:** Inhibition of TNF- $\alpha$  converting enzyme (TACE), which involves in production of mature TNF- $\alpha$ , resulted in improvement in glucose and insulin levels in diabetic animals, suggesting a crucial role of TACE activity in glucose metabolism. However, the tissue responsible for TACE activation is largely unknown.

**Materials and methods:** In this study, we investigated the impact of visceral adipose tissue (VAT) as a target of TACE activation. The expression and activity of TACE in C57BL/6 mice fed standard chow (SC) or high-fat diet (HF), KK and KKAY mice fed ad-libitum (KK-AL and AY-AL) or fed reduced amounts of SC (caloric restriction (CR); AY-CR) were studied by RT-PCR, western blot and TACE activity with a FRET analysis. Pharmacological inhibitors for JNK (SP600125; SP) and PKC (Go6983; Go) were used to investigate the signaling pathways.

**Results:** Compared with C57BL/6 mice fed SC, mice fed HF developed obesity and showed higher expression and activity of TACE. AY-AL mice were heavier and showed higher expression and activity of TACE in VAT compared with those of KK-AL mice. HF-fed C57BL/6 and AY-AL mice showed elevated phosphorylation of JNK and PKC in VAT compared with the controls. AY-CR mice had less body weight, lower glucose and insulin levels, and showed lower expression and activity of TACE in VAT compared with AY-AL mice, which were associated with reduced phosphorylation of the kinases. In in-

vitro study using 3T3-L1 adipocytes, TACE expression and activity increased 2–3-fold either by TNF- $\alpha$  or LPS stimulation, and pretreatment with SP or Go partially suppressed these events. Intraperitoneal injection of SP strongly reduced the TACE activity in VAT, and suppressed both JNK and PKC signaling in Ay-AL mice. In addition, TNF- $\alpha$  injected intraperitoneally induced TACE expression and increased JNK phosphorylation in VAT in C57BL6 mice.

**Conclusion:** These results indicate that expression and activity of TACE in VAT are up-regulated during the development of obesity at least in part via activation of JNK and PKC, and beneficial effects of CR on obesity-induced insulin resistance might be mediated in part by inhibition of TACE activity in VAT.

## 662

### INOS activity and the formation of peroxynitrite play a key role in amplifying NF $\kappa$ B transcriptional activity via the p38 mapk kinase pathway

K. Bellmann, R. Cabana, A. Charbonneau, A. Marette;

Centre de recherche de l'Institut universitaire de cardiologie et pneumologie de Québec, Canada.

**Background and aims:** Recent studies have shown that nitric oxide (NO) and various reactive nitrogen species (RNS) modulate the NF $\kappa$ B signaling pathway in settings of cancer development and apoptosis. Nevertheless, nothing is known about the role of iNOS in modulation of NF $\kappa$ B activity in metabolic tissues involved in insulin resistance. We have previously shown that the inducible nitric oxide synthase (iNOS) plays an important role in mediating insulin resistance upon lipid challenge and that this was mediated by an iNOS-dependent tyrosine nitration of insulin signaling proteins including Akt. Moreover, we observed a strong reduction of insulin-mediated glucose disposal after treatment with LPS during euglycemic-hyperinsulinemic clamps, which was completely abrogated in mice lacking iNOS. This protection was associated with improved insulin-mediated suppression of hepatic glucose production and increased liver Akt activation in iNOS-KO mice underlining the important role of iNOS in hepatic insulin resistance. Here, we evaluated whether iNOS or the RNS peroxynitrite (ONOO-) can modulate hepatic NF $\kappa$ B activation in in vitro inflammatory settings via the MAP kinase p38.

**Materials and methods:** The rat hepatic cell line (Fao) was treated with a cytokine mixture (TNF $\alpha$ , IFN $\gamma$ , IL-1 $\beta$ ) in the presence of ONOO- or the selective iNOS inhibitor 1400W to block inflammatory iNOS induction. The activation of the NF $\kappa$ B pathway was evaluated in total cell lysates and the nuclear translocation of RelB/p50 as well as its transcriptional activity were analyzed.

**Results:** Treatment of Fao cells with cytokines led to increased phosphorylation of IKK, RelB and to the degradation of I $\kappa$ Ba though this was not at all affected in the presence of ONOO- or in by iNOS inhibition using 1400W. Analysis of nuclear events revealed that ONOO- increased NF $\kappa$ B nuclear transcription factor activity by 50% as well as NF $\kappa$ B-driven luciferase activity by 20%, both of which were reduced by 1400W by 20%. Cytokine induced nuclear translocation of RelB or p50 were not affected by ONOO- or 1400W. Hence, we evaluated their effects on p38 MAP kinase activation known to play a role on the binding of the transcription coactivator p300 to NF $\kappa$ B. Interestingly, we observed a 2fold increase of p38 phosphorylation after treatment with cytokines in the presence of ONOO- and a reduction of p38 phosphorylation in the presence of the iNOS inhibitor 1400W by 21%. Furthermore, NF $\kappa$ B driven luciferase activity was reduced by 30% when p38 activity was inhibited.

**Conclusion:** We conclude that iNOS induction contributes to hepatic NF $\kappa$ B activation via formation of peroxynitrite by affecting p38 phosphorylation suggesting that this feedforward mechanism may contribute to exacerbate inflammation, thereby further impairing insulin signaling and hepatic insulin action.

Supported by: CIHR

## 663

### CD40 and costimulatory molecules B7.1, B7.2 in adipocytes-leukocytes interactions

A. Chatzigeorgiou, T. Chavakis;

Department of Internal Medicine III and Institute of Physiology, Dresden University of Technology, Germany.

**Background and aims:** Macrophages and lymphocytes are implicated in obesity-related adipose tissue inflammation and may interact with adipocytes.

The costimulatory systems CD40-CD40L and B7.1/B7.2-CD28 are essential for T cell activation and inflammatory reactions, yet the contribution of these pathways in the intercellular communications between inflammatory cells and adipocytes during adipose tissue inflammation remains unclear. Here, we assessed expression and function of these costimulatory systems in the interactions between adipocytes and lymphocytes and macrophages.

**Materials and methods:** Differentiated 3T3-L1 adipocytes and bone marrow derived macrophages (BMDM) were treated with palmitate and TNF and also with CD40L and the expression of CD40, B7.1, B7.2, chemokines and adhesion molecules was evaluated with qPCR. Migration of bone marrow mononuclear cells (BMMNCs) towards supernatants from adipocytes pre-treated with TNF and CD40L, as compared to control supernatants, was assessed. The contribution of CD40, B7.1 and B7.2 and their ligands to the adhesion between adipocytes and CD3+ T cells was evaluated.

**Results:** TNF upregulates the expression of CD40, B7.2, MCP-1, CCL3, CCL4, CCL5, ICAM-1 and VCAM-1, whereas palmitate increases the expression of CCL4 and ICAM-1 on adipocytes. CD40, B7.1, B7.2, MCP-1, CCL3 and CXCL2 are overexpressed on BMDMs upon stimulation with palmitate (without or together with TNF), although TNF alone did not upregulate the expression of these genes. Stimulation of adipocytes with CD40L for 4 hours increased the expression of CD40, MCP-1 and CCL4 (1,62 $\pm$ 0,59, 1,92 $\pm$  0,73 and 1,84 $\pm$  0,84 fold change respectively) and for 24 hours the expression of MCP-1, CCL4, CCL5 (3,11 $\pm$ 0,96, 2,43 $\pm$ 0,43 and 2,52 $\pm$ 0,63 fold change respectively). Supernatants of CD40L-pretreated adipocytes induced elevated migration of BMMN cells, as compared to control supernatants (147,83%  $\pm$  8,26%). In contrast, CD40, B7.1 and B7.2 did not contribute to the adhesion between adipocytes and CD3+ T cells.

**Conclusion:** The inflammation-dependent upregulation of CD40 on adipocytes in the adipose tissue and/or the recruitment of CD40L-expressing T cells can stimulate chemokine expression in adipocytes, thereby mediating macrophage accumulation in the adipose tissue. Macrophages in turn are characterized by lipid-mediated CD40-, B7.1- and B7.2-overexpression, a phenomenon that may contribute to perpetuation of adipose tissue inflammation.

Supported by: DFG

## PS 047 Lipid and inflammatory signalling in skeletal muscles

664

### High fat diet impairs glucose tolerance but not mitochondrial function in skeletal muscle of C57Bl/6J mice

K. Haas<sup>1,2</sup>, M. Aichler<sup>3</sup>, M. Conti<sup>4</sup>, S. Loric<sup>5,6</sup>, A. Walch<sup>7</sup>, H. Daniel<sup>8</sup>, M. Klingenspor<sup>1</sup>;

<sup>1</sup>Molecular Nutritional Medicine, Technical University Munich, Freising, Germany, <sup>2</sup>Institute for experimental genetics, Helmholtz Center Munich, Neuherberg, Germany, <sup>3</sup>Research Unit for Analytical Pathology, Helmholtz Center Munich, Neuherberg, Germany, <sup>4</sup>Clinical Biochemistry Laboratory, APHP Bicêtre University Hospital, Le Kremlin-Bicêtre, France, <sup>5</sup>Clinical Biochemistry and Genetics Laboratory, APHP Henri Mondor University Hospital, Créteil, France, <sup>6</sup>INSERM Unit 955, Faculté de Médecine, Créteil, France, <sup>7</sup>Research Unit Analytical Pathology, Helmholtz Center Munich, Freising, Germany, <sup>8</sup>Molecular Nutrition Unit, Technical University Munich, Freising, Germany.

**Background and aims:** Oxidative stress and mitochondrial dysfunction have been suggested as major contributors to the ageing process as well as the development of insulin resistance and type 2 diabetes. Mitochondria are controversially discussed as the missing link between chronic overnutrition and the loss of insulin sensitivity in peripheral tissues. In this study we used diet-induced obesity in juvenile and adult male C57Bl/6J mice as a model to analyse mitochondrial morphology, respiratory capacity and redox status in the skeletal muscle.

**Materials and methods:** Male C57Bl/6J mice either at 8 or 52 weeks of age received a high fat diet (48% kcal from fat mainly from palm-oil) or a standard diet (12% kcal from fat) for 9 weeks. Blood glucose was monitored during ipGTT. Plasma samples were prepared for insulin measurements (ELISA). A mitochondrial fraction was isolated from skeletal muscle. An array of markers for mitochondrial activity, oxidative stress and redox status was analysed in m. gastrocnemius using a commercial analytical platform. Mitochondrial morphology was investigated in m. gastrocnemius of young mice prepared for electron microscopy. Statistical analysis was performed using 2-way ANOVA.

**Results:** Robust body weight gain and progression in glucose intolerance together with elevated fasting insulin levels was seen in both age groups on high fat diet. Diet and age-related effects on cellular antioxidative mechanisms were only observed in the glutathione redox system. High fat diet elevated the GSSG levels and accordingly glutathione reductase which was generally more active in aged mice compared to young. Malondialdehyde, an indirect marker of oxidative stress, was neither affected by age nor by diet. Enzymatic activity of the respiratory chain complexes and energy charge was unchanged in all groups. Hydrogenperoxide levels measured in isolated mitochondria respiring on fatty acids increased with age but were not influenced by high fat diet in young mice and even tended to be reduced in high fat fed aged animals compared to controls. Morphology of skeletal muscle mitochondria in young animals was normal.

**Conclusion:** C57Bl/6J mice are widely used to study obesity with changes in glucose homeostasis and insulin secretion. We investigated effects of age and a high fat diet on the mitochondrial morphology and metabolism in skeletal muscle. The dietary challenge led to a constitutive change in glucose tolerance but did not reveal major metabolic lapse. Adaption of the glutathione redox system seems to sufficiently quench ROS released by mitochondria in aged animals in order to maintain full functionality. These results argue against the hypothesis that mitochondrial “dysfunction” is responsible for deleterious effects on insulin action in skeletal muscle.

Supported by: DZD, BMBF, Bioquantia

665

### Age- and genetic background-dependent inducibility of lipid catabolism in skeletal muscle by high-fat diet and by leptin in mice

J. Hansikova, V. Kus, Z. Macek Jilkova, P. Janovska, J. Kopecky; Department of Adipose Tissue, Institute of Physiology Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic.

**Background and aims:** Obesogenic effect of dietary lipids is counterbalanced by stimulation of energy expenditure and lipid oxidation. We have described

recently that in obesity-resistant A/J mice, but not in obesity-prone C57Bl/6 (B/6) mice, feeding corn oil-based high-fat diet (cHF; lipids ~35% wt/wt) for 2 weeks after weaning resulted in the induction of lipid catabolism and non-shivering thermogenesis in the oxidative skeletal muscle, in correlation with changes in leptinemia. These results suggested a role for muscle nonshivering thermogenesis and lipid oxidation in the obesity-resistant phenotype of A/J mice and indicated that cHF diet could induce thermogenesis in oxidative muscle, possibly *via* the leptin-AMPK axis. The aim of the present study was to establish whether the induction of lipid catabolism by cHF diet in skeletal muscle could be also observed in adult mice.

**Materials and methods:** Male mice of the B/6 and A/J strains were maintained at temperature closed to thermoneutrality (30 °C). Both after weaning at 4 weeks of age and at 3 months of age (adult mice; mice weaned to chow diet), mice were randomly assigned to either chow or cHF diet. After 2 weeks of the differential dietary treatment, mice of each subgroup were injected either by leptin (6 µg/g of body weight) or by saline (at 8.00 a.m.; *n*=8). After 5 hours (at 1.00 p.m.), animals were anesthetized by pentobarbital and soleus muscle was used for the measurement of the rate palmitate oxidation using [<sup>1-14</sup>C]-palmitate, and cervical blood was collected for assessment of leptinaemia.

**Results:** In accordance with our previous study (see above), cHF diet could increase 1.5-fold palmitate oxidation in the soleus muscle in A/J (cHF, 322±19 vs. chow, 214±28 dpm/mg tissue; *p*<0.05) but not in B/6 mice (cHF, 289±41 vs. chow, 309±33 dpm/mg tissue). In response to the leptin injection, this activity was increased further in A/J mice (cHF, 412±49 vs. chow, 275±16 dpm/mg tissue; *p*<0.05). In contrast, in B/6 mice, palmitate oxidation was not affected (cHF, 362±28 vs. chow, 283±24 dpm/mg tissue). In adult A/J mice, no stimulation of palmitate oxidation by either cHF or by leptin was detected. In these mice, AMPK activator AICAR stimulated palmitate oxidation in the muscle about 1.3-fold, independent on the diet. In adult mice of both strains, higher leptinaemia was detected in the cHF-fed as compared with the chow-fed mice, and leptin levels were lower in A/J than in B/6 mice (A/J, cHF, 9.39±0.82 vs. chow, 3.84±0.46ng/ml; *p*<0.05; and B/6, cHF, 34.39±6.77 vs. chow, 5.51±0.90 ng/ml; *p*<0.05).

**Conclusion:** Dietary lipids could induce thermogenesis in oxidative muscle in the post-weaning but not in adult A/J mice. Our results suggest that induction of muscle nonshivering thermogenesis by dietary fat depends on muscle leptin sensitivity, which deteriorates with advanced age, depending on the genetic background of the mice.

Supported by: EU FP7 project n° 244995 (BIOCLAIMS)

666

### Acute effects of monounsaturated fat on postprandial lipaemia and gene expression in first degree relatives of subjects with type 2 diabetes

S. Gregersen<sup>1</sup>, A. Pietraszek<sup>1</sup>, S.B. Pedersen<sup>1</sup>, J.J. Holst<sup>2</sup>, K. Hermansen<sup>1</sup>;

<sup>1</sup>Endocrinology and Metabolism, Aarhus University Hospital, <sup>2</sup>The Novo Nordisk Foundation Center for Basic Metabolic Research, Panum Institute, Copenhagen University, Denmark.

**Background and aims:** Subjects with type 2 diabetes (T2D) and their first degree relatives (REL) have increased risk of cardiovascular disease (CVD). Postprandial triglyceridaemia (PPL), influenced by diet, is an independent risk factor for CVD. Dietary fat elicits increased PPL in T2D compared with healthy controls (CON), but our knowledge on PPL responses to fat in REL is sparse. Our aim was to test the hypothesis that REL respond to a monounsaturated fatty acids (MUFA)-challenge with larger PPL than CON and that MUFA exert a differential impact on incretin responses and on expression of genes involved in carbohydrate and lipid metabolism in muscle and adipose tissue of REL and CON.

**Materials and methods:** 17 REL and 17 CON consumed a meal with 72 energy percent from MUFA (macadamia nut oil). Plasma triglycerides, free fatty acids, insulin, glucose, glucagon-like peptide 1, glucose-dependent insulinotropic peptide and ghrelin were measured at baseline and regular intervals until 4 h postprandially. Muscle and adipose tissue biopsies were taken at baseline and 210 min after the meal. Gene expression was determined using a real-time qPCR system.

**Results:** The MUFA-rich meal did not elicit different responses in PPL, insulin, glucose, incretins or ghrelin between REL and CON. Several genes were differentially regulated in muscle and adipose tissue of REL and CON. In muscle, several genes were up-regulated in CON in response to the MUFA-rich meal: *CD36*, *UCP3*, *PNPLA2* and *NR1H3*. In adipose tissue, *UCP3*, *PIK3R1*, *PRKCD*, *PRKQ*, *HK2* and *PPARGC1A* were up-regulated in response to the meal in CON, while *PDP1* was up-regulated in REL. The changes from base-



line till 210 min in expression of *ACSL1* and *PRKCD* in adipose tissue did not reach significance within groups, but differed significantly between REL and CON.

**Conclusion:** A MUFA-rich meal elicits similar PPL, insulin and incretin responses in REL and CON. MUFA has differential impact on gene expression in muscle and adipose tissue in a pattern pointing towards early defects in lipid metabolism in REL.

*Clinical Trial Registration Number:* CERN-PPDysMet-AP

*Supported by:* DanORC/SYSDIET

## 667

### Determining the role of skeletal muscle macrophages in type 2 diabetes

D. Patsouris, J. Cao, A. Durand, M.-A. Chauvin, M.-C. Michalski, H. Vidal, J. Riessset;

Inserm U1060, Oullins, France.

**Background and aims:** Type 2 diabetes (T2D) is nowadays considered a low grade inflammatory disease characterized by adipose tissue macrophages (ATMs) accumulation. Whereas it is established that secretion of pro-inflammatory cytokines by ATMs interferes with insulin signaling, this is skeletal muscle tissue which is responsible for most of whole body glucose disposal. However so far, there is very little information describing the inflammatory status of this tissue in the context of T2D. The aim of our project was hence to determine the role of skeletal muscle inflammation in the disease.

**Materials and methods:** We used three mice models of T2D. A genetic model (ob/ob), and two nutritional models. The first nutritional model consisted in feeding mice with a high fat diet (HFD, 45%) in absence or presence of rosiglitazone, an insulin sensitizing agent. The second nutritional model was mice fed with specific fatty acids such as palmitate (20%), an established saturated fatty acid with diabetogenic properties. In vivo delivery of adenovirus was realized by injecting viral particles with a needle in gastrocnemius of wild-type mice. In vitro chemotaxis assays were performed with macrophages cells exposed to myotubes conditioned media.

**Results:** we observed a striking correlation between intra-muscular pro-inflammatory cytokines (TNF $\alpha$ , IL1 $\beta$ ) or macrophages markers (F4/80, CD11b, CD11c) and insulin resistance in mice. Furthermore, inflammation observed in skeletal muscles of ob/ob mice is distinct to what is observed in adipose tissue and liver. We observed that the overexpression of MCP1 in gastrocnemius of wild-type mice was sufficient to alter insulin signaling in this tissue, which was correlated with an elevation inflammation. Finally, in vitro chemotaxis assays demonstrated that skeletal muscle macrophages might be recruited in response to the secretion of chemoattractants (MCP1) by myotubes exposed to excess fatty acids.

**Conclusion:** Our study indicates that T2D is associated with the recruitment of pro-inflammatory macrophages in skeletal muscle tissue, which may in turn induce or worsen the disease. This macrophages may be recruited in response to excess delivery of saturated fatty acids to myotubes, which may in turn induce the expression of chemokines such as MCP1.

*Supported by:* Agence Nationale pour la Recherche (ANR-09-RPDOC-018-01)

## 668

### Macrophage-conditioned medium impairs insulin sensitivity in C2C12 myotubes through activation of mitogen-activated protein kinases

N.A. Talbot, C.P. Wheeler-Jones, M.E. Cleasby;

Comparative Biomedical Sciences, Royal Veterinary College, London, UK.

**Background and aims:** Skeletal muscle is the principal site for insulin-stimulated glucose disposal but its ability to utilise glucose is diminished by obesity-associated insulin resistance (IR) and the related elevation of plasma fatty acids (FA). Chronic sub-clinical inflammation, characterised by increased macrophage infiltration into obese adipose tissue is a hallmark of the insulin resistance syndrome. However, it is not known whether macrophages might play a role in mediating the local effects of elevated FA levels in muscle. Here we aimed to determine whether macrophage-conditioned medium would impair glucose disposal by cultured myotubes and the mechanisms involved.

**Materials and methods:** J774 macrophages (m $\phi$ ) were incubated with or without saturated FA (palmitic acid), unsaturated FA (palmitoleate) or lipopolysaccharide (LPS) for 8 hours to generate conditioned medium. Differentiated C2C12 myotubes were incubated with m $\phi$ -conditioned medium for 16 hours and the effects on intracellular signalling and glycogen synthesis assessed. In separate experiments, myotubes were pre-incubated for 1 hour

with DMSO, p38 MAPK inhibitors SB203580 (1 $\mu$ M) or BIRB796 (0.1 $\mu$ M), these inhibitors in combination, or JNK inhibitor V (1 $\mu$ M), prior to treatment with conditioned medium and assessment of the effects on signalling. 3-5 independent experiments were conducted in each case.

**Results:** Incubation of myotubes with palmitic acid-treated m $\phi$ -conditioned medium caused a 53% reduction in insulin-stimulated glycogen synthesis ( $p < 0.01$ ), accompanied by reductions in phosphorylation of PI3-kinase pathway intermediates of 57% for IRS-1 (Tyr612;  $p < 0.07$ ), 53% for Akt (Ser473;  $p < 0.005$ ), 56% for AS160 (Thr642;  $p < 0.05$ ) and 65% for GSK3 $\beta$  (Ser9;  $p < 0.01$ ). The conditioned medium activated MAP kinases, indicated by a 110% increase in JNK2 ( $p < 0.001$ ) and a 91% increase in p38 (Tyr182;  $p < 0.001$ ) phosphorylation. In addition, I $\kappa$ B $\alpha$  protein was reduced by 42% ( $p < 0.05$ ), together implying activation of stress/inflammatory pathways in myotubes. These changes were mirrored by the effects of LPS-conditioned medium. In contrast, palmitoleic acid-treated m $\phi$ -conditioned medium enhanced glycogen synthesis by 36% and increased phosphorylation of IRS-1, AS160 and GSK3 $\beta$ . When added in combination, palmitoleic acid-conditioned medium negated the effect of palmitic acid on signalling. To establish whether MAPK activation mediated the inhibitory effect of palmitic acid-treated m $\phi$ -conditioned medium on insulin signalling, conditioned medium was added to myotubes in the presence of specific inhibitors. Tyr612 phosphorylation of IRS1 was partially ameliorated by the addition of SB203580, SB203580 and BIRB796 in combination and by JNK inhibitor V. Inhibition of p38 MAPK using BIRB796 alone corrected 93% of the defect in IRS-1 phosphorylation. The defect in GSK3 $\beta$  Ser9 phosphorylation was ameliorated by the addition of both p38 MAPK and JNK inhibitors. The combination of SB203580 and BIRB796 corrected 80% of the defect in GSK3 $\beta$  phosphorylation induced by palmitic acid-treated m $\phi$ -conditioned medium.

**Conclusion:** These findings demonstrate that macrophage-derived factors may play a direct role in mediating the negative effects of elevated saturated plasma FAs on muscle insulin sensitivity. The mechanism of this effect involves inhibition of muscle insulin signalling by MAP kinases. Conversely, the presence of unsaturated FAs is protective against m $\phi$ -induced IR.

*Supported by:* Royal Veterinary College and The Royal Society

## 669

### The novel myokine YKL-40 is regulated by inflammatory cytokines and partially prevents TNF $\alpha$ induced pro-inflammatory signalling in primary human skeletal muscle cells

S.W. Görgens, K. Eckardt, J. Eckel;

Paul-Langerhans-Gruppe für Integrative Physiologie, Deutsches Diabetes-Zentrum, Düsseldorf, Germany.

**Background and aims:** The glycoprotein YKL-40 is a member of the 18 glycosyl hydrolase family, but has no chitinase activity. The lack of enzymic activity is attributed to the substitution of the catalytically essential glutamic acid residue in the active site of the glycohydrolases with a neutral amino acid. YKL-40 plays a role in tissue remodeling and inflammatory processes. Elevated serum levels of YKL-40 are found in patients with cardiovascular disease, type 2 diabetes and insulin resistance. Based on proteomic profiling of secreted proteins from primary human skeletal muscle cells (hSkMC) we identified YKL-40 as a novel myokine. However, the physiological role of YKL-40 remains largely unknown. The aim of this study was to investigate the regulation of YKL-40 in hSkMC by adipose tissue-derived factors and its potential autocrine function.

**Materials and methods:** hSkMC were *in vitro* differentiated, and RNA and protein samples were obtained every second day. Analyses of YKL-40 mRNA expression were done by quantitative RT-PCR while protein levels were analyzed using SDS-PAGE and Western Blot. In addition, supernatants were collected and analyzed for YKL-40 concentration by ELISA. Differentiated hSkMC were treated with recombinant human IFN- $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-6, and adipocyte conditioned medium (CM) for 24h. Additionally, hSkMC were acutely stimulated with TNF $\alpha$  in combination with YKL-40. Effects on YKL-40 protein level and secretion as well as on NF $\kappa$ B and ERK1/2 phosphorylation were investigated by Western Blot.

**Results:** During differentiation of hSkMC we found a continuous decrease of protein level resulting in 30% YKL-40 protein level in differentiated hSkMC compared to myoblasts. YKL-40 mRNA expression strongly decreased by ~50% at the second day of differentiation. Thereafter, mRNA expression further decreased resulting in a downregulation of 80% in myotubes compared to myoblasts. The secretion of YKL-40 follows these patterns. While the supernatant of myoblasts contains  $1.12 \pm 0.24$  ng/ml YKL-40, we found only  $0.43 \pm 0.12$  ng/ml YKL-40 in the supernatant of myotubes. After incuba-

tion with CM we observed a 3.5fold increase of YKL-40 protein level. Treatment of hSkMC with the inflammatory factors IFN- $\gamma$ , TNF $\alpha$ , IL-1 $\beta$  and IL-6 significantly increased YKL-40 protein level (~2.5fold). In addition, IFN- $\gamma$ , TNF $\alpha$ , IL-1 $\beta$  and IL-6 treatment significantly increased the secretion of YKL-40 (~2fold). Furthermore, we observed a 2fold elevated mRNA expression after incubation with TNF $\alpha$ . While no effect of YKL-40 itself on NF $\kappa$ B and ERK1/2 phosphorylation was observed, we found a ~20% reduction of TNF $\alpha$ -induced NF $\kappa$ B and ERK1/2 activation when the cells were co-incubated with TNF $\alpha$  and YKL-40.

**Conclusion:** In summary, YKL-40 is a new myokine characterized by a differentiation dependent expression and secretion. CM, that contains the secretory output of adipocytes, as well as inflammatory cytokines are able to increase YKL-40 protein level and secretion in hSkMC. Thus, we suggest that YKL-40 is a new important factor in the crosstalk between adipose tissue and skeletal muscle. It may be speculated that the upregulation of YKL-40 has a protective meaning which may involve a modification of TNF $\alpha$ -induced pro-inflammatory signalling.

*Supported by: DFG*

## PS 048 Mechanisms for adipocytes differentiation

670

### Metabolic pathways regulated during adipogenesis - potential novel biomarkers for obesity

A. Halama<sup>1</sup>, M. Horsch<sup>1</sup>, G. Kastenmüller<sup>2</sup>, G. Möller<sup>1</sup>, M. Hrabé de Angelis<sup>1,3</sup>, J. Beckers<sup>1,3</sup>, J. Adamski<sup>1,3</sup>;

<sup>1</sup>Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, <sup>2</sup>Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, <sup>3</sup>Lehrstuhl für Experimentelle Genetik, Technische Universität München, Freising, Germany.

**Background and aims:** Obesity is a major risk factor for a number of diseases like diabetes type 2, insulin resistance or hyperglycemia. The mechanisms underlying adipogenesis are not well known yet therefore a study on cell adipogenesis using metabolomics combined with transcriptomics was performed to find new biomarkers of metabolic disorders.

**Materials and methods:** The mouse 3T3-L1 cell line, a widely used model to study the mechanisms of diabetes, was chosen to monitor changes in metabolite and gene expression levels at various stages of adipogenesis (8 time points set from day 0 to day 18). Metabolites and mRNA expression were determined with the AbsoluteIDQp180 kit from Biocrates and the MouseRef-8 v2.0 Expression BeadChip kit from Illumina, respectively. Cell differentiation was induced by insulin, dexamethasone and 3-isobutyl-1-methylxanthine (IBMX), and monitored with Oil Red O assay.

**Results:** We found significantly regulated genes correlating with changes in metabolite profile which exhibits characteristic pattern for different stages of adipogenesis. The most potent transcription factors like PPAR $\gamma$ , KLF5, KLF15 or GLUT4 known to be involved in promoting adipogenesis were strongly expressed in our study. Genes with highest score were further analyzed with Ingenuity System Pathway Analysis (IPA) and linked to lipid metabolism, metabolic disease, nutritional disease and cancer. Among metabolites, significantly regulated patterns were most prominent in phospholipids, lysophosphatidylcholines and sphingomyelins. Remarkable, other metabolites like amino acids (arginine, glutamine, serine and branched chain amino acids (BCAA)), carnitines (C0, C2, C3 and C4) and biogenic amines (putrescine, spermidine and spermine) are also regulated as cells undergo adipogenesis. Evaluation of the integrated transcriptomics data indicates an involvement in several affected pathways, as BCAA degradation, citric cycle, glycolysis, and glycerophospholipid synthesis. Moreover, de novo synthesized phosphatidylcholines affects metabolism of long-chain polyunsaturated fatty acids due to increased activity of phospholipase (PLA2) and fatty acid desaturase (FADS2).

**Conclusion:** This study shows the potential of combining metabolomics and transcriptomics in research of cellular processes, like adipogenesis. Especially molecules linked to BCAA metabolism and phospholipid synthesis like phosphatidylcholines seems to play a crucial role in fat cell differentiation therefore might be potential early biomarkers in the pathogenesis of obesity or diabetes.

*Supported by: BMBF to the German Center for Diabetes Research (DZD e.V.)*

671

### Transcriptome analysis of 3T3-L1 preadipocyte differentiation using RNA-seq reveals formerly unidentified differentially regulated genes

G. Mitterer<sup>1</sup>, S. Tauber<sup>2</sup>, F. Klinglmueller<sup>3</sup>, J. Husa<sup>1</sup>, J. Lindroos<sup>1</sup>, M. Jeitler<sup>1</sup>, O. Wagner<sup>1</sup>, M. Bilban<sup>1</sup>;

<sup>1</sup>Department of Laboratory Medicine, Medical University of Vienna, <sup>2</sup>Center for Integrative Bioinformatics Vienna, Max F. Perutz Laboratories, University of Vienna, <sup>3</sup>Center for Medical Statistics, Informatics and Intelligent Systems, Medical University of Vienna, Austria.

**Background and aims:** It is generally accepted that the regulation of adipogenesis prevents obesity. However, the mechanisms controlling adipogenesis have not been completely defined. Much of our knowledge of the molecular cascade regulating adipogenesis has come from transcriptome profiling data of adipocyte differentiation generated by DNA Microarray experiments. However, expression microarrays are limited due to a high background signal, the need for known template sequences, variabilities in probe hybridization and a fairly limited dynamic range. Hence, it is a plausible that important genes involved in adipogenesis are expressed below the reliable detection lev-

els of microarrays. In contrast, deep RNA sequencing (RNA-seq) is a recently developed approach for transcriptome profiling, where the RNA/cDNA species within a sample are sequenced, that does not suffer from the limitations described for Microarrays. Our goals were to use RNA-seq (i) to identify novel regulators of adipogenesis throughout 3T3-L1 preadipocyte differentiation and (ii) to compare sequencing data to results obtained from Microarrays.

**Materials and methods:** Murine 3T3-L1 preadipocytes were differentiated into mature adipocytes with 1.7  $\mu$ M insulin, 10 mM dexamethasone, 0.5mM 3-isobutyl-1-methylxanthine and 1  $\mu$ M Troglitazone. Total RNA was extracted on days 0, 2, 4 and 6 of 3T3-L1 preadipocyte differentiation and processed for transcriptome profiling either with Affymetrix Gene Level 1.0 ST arrays or the TrueSeq paired-end mRNA protocol on a Illumina HiSeq2000 platform. Differential gene expression was estimated using either fluorescence intensities (Microarray) or RPKM values (RNA-Seq). A fold-change (FC) of  $\pm 3.0$  was used to capture differentially expressed genes (DEG).

**Results:** Overall, the RNA-Seq approach identified a larger number of  $\pm 3.0$ -fold DEG in 3T3-L1 preadipocyte differentiation as compared with Gene 1.0 ST arrays on Days 2, 4 and 6 i.e. RNASeq: 2620, 4019 and 3972 genes versus Microarray: 443, 788 and 910. Importantly, the RNA-seq approach exclusively identified a substantial number of  $\pm 3.0$ -fold DEG in comparison to Gene 1.0 ST arrays i.e. 2190, 3287 and 3106 genes. Plotting FC values of RNA-seq versus Microarray revealed a population of genes that exhibited FC values at or near zero by Microarray analysis but exhibiting a wide range of FC values by RNA-seq, highlighting the sensitivity of the RNA-seq approach. The majority of upregulated DEG identified exclusively by the RNA-seq method, involved protein-DNA interactions, mitochondrial activity and carbohydrate metabolism, whereas downregulated DEG were regulators of extracellular matrix assembly, cell adhesion and proliferation.

**Conclusion:** Transcriptome profiling of adipocyte differentiation has improved our understanding of the molecular mechanisms of adipogenesis. Although microarrays have been instrumental in this regard, it is clear that these tools detect an incomplete set of DEG. Therefore the RNA-seq approach can be used to supplement these prior technologies, which could help identify novel target genes involved in adipogenesis in disease.

## 672

### Dietary regulation of VEGFb gene expression is related to its promoter DNA methylation levels

E. Garcia-Escobar<sup>1,2</sup>, S. Garcia-Serrano<sup>1,2</sup>, S. Morcillo<sup>1,3</sup>, G.M. Martin-Nuñez<sup>1,2</sup>, E. Rubio-Martin<sup>1,2</sup>, C. Gutierrez-Repiso<sup>1,2</sup>, F. Soriguer<sup>1,2</sup>, G. Rojo-Martinez<sup>1,2</sup>;

<sup>1</sup>Ciberdem CB07/08/0019 of the Instituto de Salud Carlos III, Spain, Malaga,

<sup>2</sup>Endocrinology and nutrition service, Carlos Haya hospital, Malaga,

<sup>3</sup>Endocrinology and research group, hospital de Cruces and university of the Basque Country UPV-EHU, Barakaldo, Spain.

**Background and aims:** Studying the transmembran fatty acid transport and its regulation has awakened a big interest because of its relationship with the development of different metabolic diseases such insulin resistance, obesity or diabetes. DNA methylation is a potent regulator of gene expression, which plays a crucial role in both physiology as well as pathology. It has been recently described the implication of the vascular endothelial growth factor B (VEGFb) in the tissue lipid uptake, which may open up the possibility for novel strategies to modulate pathological lipid accumulation in obesity or type 2 diabetes. The aim of this study is investigate the effect of dietary fatty acid composition in VEGFb gene expression regulation in rat adipose tissues, and whether this effect is related by DNA methylation modification on the VEGFb promoter.

**Materials and methods:** A group of rats (4 weeks old) was assigned to one of three different isoenergetic diets, each with a significantly different concentration of saturated, monounsaturated and polyunsaturated fatty acids (coconut oil diet, olive oil diet and sunflower diet respectively). Animals were feed during a month. Samples of omental and subcutaneous adipose tissue (OAT and SAT respectively) were immediately taken for the methylation and gene expression studies, and also samples of these tissues were taken for measurements of the tissue triacylglycerol fatty acid composition, and total tissue lipid accumulation.

**Results:** Neither animal or adipose pad weights varied according to diets, nor even tissues lipid accumulation. Tissue fatty acid composition was significantly different depending on the diet consumed in both tissue ( $p < 0.0001$  for all fatty acids studied). The VEGFb gene expression level was directly correlated with the lipid accumulation of each tissue (OAT:  $r = 0.49$ ;  $p = 0.04$ . SOT:  $r = 0.58$ ;  $p = 0.01$ ) and with tissue fatty acid composition. In both tissue,

VEGFb gene expression and promoter methylation levels were different regarding to the diet consumed (OAT gene expression:  $p = 0.002$ . SAT gene expression:  $p = 0.01$ . OAT promoter methylation:  $p = 0.005$ . SAT promoter methylation:  $p = 0.01$ ), being VEGFb gene expression and promoter methylation levels inversely correlated (OAT:  $r = -0.47$ ;  $p = 0.05$ . SAT:  $r = -0.56$ ;  $p = 0.03$ ), with the highest values of gene expression, and the lowest of promoter methylation, found in rats feed with coconut oil diet.

**Conclusion:** VEGFb gene expression in adipose tissue is associated with the increase in fat accumulation. The presented study shows that VEGFb gene expression is regulated by dietary fatty acid, and this regulation is related to the methylation level of VEGFb promoter region.

*Supported by: ISCIII(11/00880; 09/02117) and Junta de Andalucía (0532-2010)*

## 673

### A microdeletion within an obesity QTL on distal mouse chromosome 1 disrupts the *Ifi202b* gene which modulates preadipocyte proliferation

H. Vogel, T. Kanzleiter, S. Scherneck, V. Benz, R. Kluge, H.-G. Joost, A. Schürmann;

Experimental Diabetology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany.

**Background and aims:** The New Zealand Obese (NZO) mouse develops a polygenic disease pattern of obesity, insulin resistance, and dyslipoproteinemia resembling the human metabolic syndrome. By positional cloning we have recently discovered two genes (*Tbc1d1*, *Zfp69*) to participate in the development of the disease. In an outcross population of NZO and lean C57BL/6 (B6) mice we identified a major obesity QTL on distal chromosome 1 responsible for a body weight increment of 13 g in week 22.

**Materials and methods:** The conventional strategy of positional cloning, including generation of congenic lines with the lean B6 background, was used to reduce the critical fragment size and to characterize the phenotype of the QTL *Nob3* in more detail. Genes within the critical region were finally analyzed by sequencing and expression profiling.

**Results:** Introgression of a 38 Mbp segment of the QTL from NZO into B6 (B6.NZO-*Nob3*.38) increased body weight and fat mass. We generated additional congenic lines, tested them for the trait body weight and defined a genomic interval comprising 43 genes. Expression analysis of genes located in the critical fragment revealed the most striking difference for the gene *Ifi202b*, encoding for a transcriptional regulator. In tissues of homozygous B6-animals *Ifi202b* was undetectable but expressed in NZO mice. In contrast, expression of 10 related genes (*Ifi200* cluster) was significantly higher in B6 than in NZO-allele carriers. Consistent with a regulation of the cluster by *Ifi202b*, overexpression of its mRNA in skeletal muscle of B6 mice suppressed the expression of other genes within the cluster. Highest expression levels of *Ifi202b* were found in white adipose tissue. Suppression of *Ifi202b* in 3T3-L1 fibroblasts induce proliferation as detected by *Ki67* and *Pcna* expression. Sequence analysis identified a microdeletion including the first exon and the 5'-flanking region of the *Ifi202b* gene in B6.

**Conclusion:** We conclude that a microdeletion on chromosome 1 causes a loss of function of the *Ifi202b* gene in the B6 strain, affects the expression of other family members, and is responsible for decreased proliferation of preadipocytes leading to the obesity-suppressing phenotype of the B6 strain.

## 674

### Bone marrow fat: relationship with adiposity, insulin sensitivity, leptin and adiponectin

F. Amati<sup>1</sup>, Y. Sheu<sup>2</sup>, B.H. Goodpaster<sup>2</sup>, T. Prasad<sup>2</sup>, M.E. Danielson<sup>2</sup>, A.V. Schwartz<sup>3</sup>, J.A. Cauley<sup>2</sup>;

<sup>1</sup>University of Lausanne, Switzerland, <sup>2</sup>University of Pittsburgh, USA,

<sup>3</sup>University of California, San Francisco, San Francisco, USA.

**Background and aims:** Osteoporosis has been linked with lower bone marrow fat (BMF). With advancing age, differentiation of mesenchymal stem cells (MSCs) favors adipocytes instead of osteoblasts. Studies have found that lumbar bone mineral density (BMD) has an inverse relationship with BMF content. A positive association between visceral adipose tissue (VAT) and BMF has recently been shown in obese women, as well as a positive correlation between BMF and HbA1c in diabetic women. To our knowledge, no studies look at the relationship between BMF and regional fat depositions in men, or insulin resistance. Furthermore, a possible mechanistic explanation of the



impact of VAT on BMF could be the fact that MSCs are under the influence of adipokines such as leptin and adiponectin. Thus the objectives of this study were to observe the relationships of BMF with 1) VAT, abdominal subcutaneous adipose tissue (aSAT), calf subcutaneous (cSAT) and intermuscular (IMAT) adipose tissue, 2) markers of insulin resistance and 3) serum leptin and adiponectin in older men.

**Materials and methods:** Non-diabetic men were recruited for this study. BMF was measured by proton magnetic resonance spectroscopy in the lumbar vertebrae (L1–L3); percent body fat (BF) by dual-energy X-ray absorptiometry. VAT, aSAT, cSAT and IMAT were assessed by quantitative computed tomography. Fasting blood sugar (FBG) was measured enzymatic method; insulin, leptin and adiponectin were measured by RIA.

**Results:** 149 non diabetic men aged 74–95 years old participated in this study. Mean BMI was  $28.0 \pm 3.7$  kg/m<sup>2</sup> (range 18.8–38.4), mean BMF was  $55.5 \pm 11.1$ % (23.5–76.4) and mean BF was  $27.8 \pm 4.8$ % (17.5–40.9). Average BMF (L1–L3) was correlated with BF ( $r=0.35$ ,  $P=0.01$ ) and VAT ( $r=0.34$ ,  $P=0.01$ ), but was not correlated with BMI, aSAT, cSAT or IMAT. The relationship between BMF and VAT remained after controlling for BMI. After adjusting for age, BMF was associated with overall adiposity, VAT and leptin, but not with FBG, insulinemia, HOMA-IR or adiponectin (table).

**Conclusion:** The positive relationship between BMF and VAT observed in women is also true in older men. BMF was positively correlated with plasma leptin, but not with adiponectin or markers of insulin resistance. Further studies are needed to explore the relationship between VAT and BMF, particularly the hormonal influence of adipokines on BMF.

Table : Age-adjusted means by BMF (%) quartiles

	Q1 N=38	Q2 N=37	Q3 N=37	Q4 N=37	P for trend
BMI (kg/m <sup>2</sup> )	28.02	27.59	27.91	28.39	0.601
Fat mass (g)	22193.92	22393.34	23428.34	24959.43	<b>0.047</b>
Body fat %	27.04	26.88	27.84	29.30	<b>0.026</b>
Waist (cm)	99.79	100.88	103.88	103.82	<b>0.039</b>
VAT volume (cm <sup>3</sup> )	49.33	62.41	52.82	70.79	<b>0.039</b>
FBG (mg/dl)	109.21	103.16	103.63	114.56	0.258
Insulin (uU/ml)	14.81	16.21	14.06	15.78	0.862
HOMA-IR	4.01	4.16	3.61	4.51	0.464
Leptin (ng/ml)	10.41	10.91	12.19	13.30	<b>0.035</b>
Adiponectin (ug/ml)	12.39	10.63	13.54	12.00	0.703

\* BMF (%) quartile cut-points: 47.90 %, 54.90% and 63.86%

Supported by: NIH

## 675

### Enhanced adipo/lipogenic potential of human adipose tissue stem cells in obesity

S. Perrini<sup>1</sup>, P. Nigro<sup>1</sup>, A. Cignarelli<sup>1</sup>, M. Barbaro<sup>1</sup>, R. Ficarella<sup>1</sup>, A. Peschechera<sup>1</sup>, M. De Fazio<sup>2</sup>, F. Puglisi<sup>2</sup>, A. Natalicchio<sup>1</sup>, L. Laviola<sup>1</sup>, F. Giorgino<sup>1</sup>;

<sup>1</sup>Endocrinology & Metabolic Diseases, University of Bari, <sup>2</sup>General Surgery and Liver Transplantation, University of Bari, Italy.

**Background and aims:** An impaired adipo/lipogenic capacity of human adipose stem cells (ASCs) may contribute to obesity and the related metabolic abnormalities. Aim of this study was to investigate whether obesity is associated with changes in the adipogenic and/or lipogenic potential of ASCs from the subcutaneous (SC) and visceral (V) fat tissue depots.

**Materials and methods:** ASCs were isolated from SC and V fat biopsies, obtained from 8 lean (L) (BMI  $24.1 \pm 2.3$  kg/m<sup>2</sup>) and 8 obese (Ob) (BMI  $37.4 \pm 3.2$  kg/m<sup>2</sup>) subjects. Cells were differentiated in vitro with adipogenic inducers in the absence or presence of 100 nM insulin. Staining with Nile Red or Oil-Red-O was used to quantify adipocyte conversion and the lipid droplet size and number, respectively. mRNA levels of lipogenic and adipogenic genes were measured by qRT-PCR.

**Results:** No significant differences in the conversion to mature adipocytes were seen in SC-ASCs from L and Ob subjects, whereas adipogenesis was about 60% higher in V-ASCs from Ob compared to L donors. Following differentiation of both V-ASCs and SC-ASCs into mature adipocytes in the absence of insulin, there was a 70–80% higher number of lipid droplets, with no

apparent change in size, in Ob compared to L cells ( $p<0.05$ ). Similar results were obtained in the presence of insulin, and insulin-induced Akt and Erk-1/2 phosphorylation was similar in L and Ob ASCs. When the expression of 14 lipogenic and adipogenic genes was assessed in both ASCs and adipocytes from the experimental subjects, larger differences were found in V compared to SC cells ( $p<0.05$ ). Specifically, the SIRT1/2 mRNA levels were lower in Ob compared to L V-ASCs and adipocytes. A trend to an inverse relationship between SIRT1/2 mRNA expression and BMI was also found in V fat biopsies ( $R= -0.145$ ).

**Conclusion:** In conclusion, Ob ASCs, particularly from the V fat depot, exhibit lower SIRT1/2 gene expression and possess an enhanced inherent adipo/lipogenic potential, which is largely insulin-independent.

Supported by: Fo.Ri.SID, Italy

## 676

### Decreased PRDM16 gene expression in subcutaneous and visceral human adipose tissue in association with obesity

E. Esteve, J. Moreno-Navarrete, F. Ortega, M. Serrano, G. Pardo, N. Pueyo, E. Guerra, W. Ricart, J. Fernández-Real; Hospital of Girona, Spain.

**Background and aims:** Recent studies in mice have shown that Prdm16, a transcriptional modulator of thermogenic genes in brown adipose tissue, was expressed at substantial levels in subcutaneous WAT, promoting the expression of brown fat-specific genes in this fat depot. We aimed to investigate the possible role of PRDM16 in human adipose tissue, we aimed to analyze its expression in association with obesity, type 2 diabetes and insulin sensitivity.

**Materials and methods:** PRDM16 gene expression (Real Time PCR) was analyzed in two independent cohorts: in participants with different degrees of obesity (88 visceral and 83 subcutaneous adipose tissue samples) and in a second cohort of morbidly obese participants with different degrees of insulin action (measured with euglycemic clamp) (32 paired visceral and 32 subcutaneous adipose tissue samples).

**Results:** In the first cohort, PRDM16 gene expression was detected in both subcutaneous and visceral adipose tissues at similar levels. PRDM16 gene expression was significantly decreased in obese patients. In subcutaneous adipose tissue, PRDM16 gene expression was negatively associated with BMI ( $r= -0.23$ ,  $p= 0.04$ ), fasting glucose ( $r= -0.27$ ,  $p= 0.01$ ), and positively with the expression of IRS1 ( $r= 0.55$ ,  $p< 0.0001$ ) and mitochondrial genes [PGC1 $\alpha$  ( $r= 0.46$ ,  $p< 0.0001$ ) and mt-CO3 ( $r= 0.27$ ,  $p= 0.01$ )]. In visceral adipose tissue, PRDM16 gene expression was negatively associated with BMI ( $r= -0.30$ ,  $p= 0.005$ ), and positively with PPAR $\gamma$  ( $r= 0.40$ ,  $p= 0.004$ ), GLUT4 ( $r= 0.39$ ,  $p= 0.004$ ), IRS1 ( $r= 0.49$ ,  $p< 0.0001$ ), PGC1 $\alpha$  ( $r= 0.43$ ,  $p< 0.0001$ ) and mt-CO3 ( $r= 0.55$ ,  $p< 0.0001$ ). In multiple linear regression analysis, IRS1 and PGC1 $\alpha$  and mt-CO3 contributed independently to PRDM16 gene expression after controlling for BMI in both visceral and subcutaneous adipose tissues. In the second cohort (morbidly obese patients), PRDM16 was not associated with insulin sensitivity. The correlations with GLUT4, IRS1, PGC1 $\alpha$  and mt-CO3 were replicated. Interestingly PRDM16 in visceral adipose tissue was significantly and negatively associated with glycated hemoglobin.

**Conclusion:** The strong association among PRDM16, mitochondrial genes and IRS1 suggests that PRDM16 is a potential marker of brown-like adipose tissue in human fat similar to mouse fat. In this sense, brown-like characteristics of adipose tissue decrease with obesity.

Supported by: ISCIII and CIBERobn

## 677

### Visualisation of brown adipose tissue in humans with <sup>123</sup>I-metaiodobenzylguanidine SPECT-CT and <sup>18</sup>F-fluorodeoxyglucose PET-CT

W.M. Admiraal<sup>1</sup>, H.J. Verberne<sup>2</sup>, L. Bähler<sup>1</sup>, J.B.L. Hoekstra<sup>1</sup>, F. Holleman<sup>1</sup>; <sup>1</sup>Internal Medicine, Amsterdam, <sup>2</sup>Nuclear Medicine, Academic Medical Centre, Amsterdam, Netherlands.

**Background and aims:** Given its high capacity to burn excess energy, brown adipose tissue (BAT) has become a focus of research in the hope that activation of BAT may prove to be a new target to fight obesity. However, knowledge of mechanisms regulating BAT activity is only slowly progressing. For research purpose in humans, BAT is visualized with an <sup>18</sup>F-fluoro deoxyglucose (FDG) positron emission tomography (PET) computed tomography (CT). Results from studies performed in animals show that thermogenesis

in BAT is influenced by the sympathetic nervous system. In nuclear medicine,  $^{123}\text{I}$ -metaiodobenzylguanidine (MIBG), a non-functional noradrenalin analog, is used for scintigraphic assessment of neuroendocrine tumors and cardiac sympathetic activity. Therefore, given the strong sympathetic innervation of BAT, MIBG scintigraphy has been used to localize BAT in rats. In humans,  $\beta$ -adrenergic antagonists eliminate glucose uptake into BAT, suggesting that, also in man, thermogenesis is under  $\beta$ -adrenergic control. The aim of this study was to determine whether MIBG single photon emission CT (SPECT-CT), as a measure of sympathetic stimulation, and FDG PET-CT identify the same anatomical locations as BAT in adult humans. Furthermore, we investigated whether the magnitude of BAT activity measured by these two techniques correlated.

**Materials and methods:** We studied 10 lean (BMI 18–24), healthy, Caucasian males, 18–32 years old. All underwent MIBG SPECT-CT and FDG PET-CT within a 2-week interval and in random order. On both occasions, the subjects were exposed to mild cold ( $17^\circ\text{C}$ ) for the duration of 2 hours after an overnight fast. After 1 hour of cold exposure, FDG or MIBG was administered intravenously. MIBG imaging was performed 24 hours after administration, whereas the FDG PET-CT was performed 1 hour after administration. Activity of BAT was defined as maximum standard uptake value ( $\text{SUV}_{\text{max}}$ ) of FDG or as semi-quantitative uptake of MIBG in the same regions of interest. These anatomical regions of interest were cervical, supraclavicular, and superior mediastinal depots. A maximal standard uptake value ( $\text{SUV}_{\text{max}}$ ) of FDG  $\geq 2.0$  g/ml was considered to indicate BAT. Using the MIBG SPECT images, semi-quantitative uptake of MIBG in BAT was calculated as the maximum count in the anatomical volumes of interest divided by the mean count per voxel in a reference region (i.e. the lung). The correlation between semi-quantitative uptake of MIBG and  $\text{SUV}_{\text{max}}$  of FDG was determined with a Pearson correlation coefficient.

**Results:** FDG uptake in BAT was visually observed in 8 out of 10 subjects, whereas MIBG uptake in BAT was observed in 7 out of 10 subjects. All subjects that showed MIBG uptake in BAT, also showed FDG uptake in BAT. Both  $\text{SUV}_{\text{max}}$  of FDG and semi quantitative uptake value of MIBG were normally distributed. A strong, positive correlation was found between the  $\text{SUV}_{\text{max}}$  of FDG and the semi-quantitative uptake of MIBG ( $r=0.65$ ,  $p=0.04$ ).

**Conclusion:** MIBG SPECT-CT, as a marker of sympathetic activity, and FDG PET-CT, as a marker of metabolic activity, identify the same anatomical regions as active BAT. Moreover, the correlation between these two techniques is strongly positive. These findings underscore that sympathetic activity is a principal driving force for BAT activity in humans.

## 678

### Macrophage infiltration and BAT distribution in adipose tissue of children

D. Rockstroh<sup>1</sup>, I. Wagner<sup>1</sup>, K. Landgraf<sup>1</sup>, R. Tauscher<sup>1</sup>, J. Gesing<sup>1</sup>, S. Weise<sup>1</sup>, M. Blüher<sup>2</sup>, W. Kiess<sup>1</sup>, H. Till<sup>3</sup>, M. Wojan<sup>4</sup>, A. Körner<sup>1</sup>;

<sup>1</sup>University Hospital for Children and Adolescents, <sup>2</sup>University Hospital for Endocrinology and Nephrology, <sup>3</sup>University Hospital for Pediatric Surgery,

<sup>4</sup>University Hospital for Orthopaedic Surgery, Leipzig, Germany.

**Background and aims:** The role of adipose tissue in the development of obesity in children has not been sufficiently investigated. In adults it has recently been shown that obesity is accompanied by an increased infiltration of macrophages. Compared to adults, children are hypothesized to have considerably more brown adipose tissue (BAT), which decreases within the first years of life. In this study we aimed to characterize the infiltration of macrophages in adipose tissue and the distribution of BAT in children.

**Materials and methods:** We analyzed 150 adipose tissue samples of lean ( $n=100$ ; mean age= 7; range= 0–20) and obese ( $n= 50$ ; mean age= 11; range= 0–18) children for macrophage infiltration and BAT distribution.

**Results:** Immunohistochemical analyses of the macrophage-specific marker CD68 indicated a positive correlation of macrophage infiltration with BMI-SDS and adipocyte size in subcutaneous depots. We detected crown-like structures in 40% of the overweight/obese, but only in 5% of the lean children. Altogether, 12 of the 150 tissue samples presented a positive UCP1 staining, which corresponded with a significantly increased *UCP1 mRNA* expression compared to WAT. Exclusively lean children aged 0–10 showed prevalence for BAT. Morphometrically, brown adipocytes of lean children were significantly smaller ( $1477\pm346\mu\text{m}^2$ ) than white adipocytes of lean ( $4316\pm254\mu\text{m}^2$ ) and overweight/obese children ( $6251\pm313\mu\text{m}^2$ ); ( $P<0.001$ ).

**Conclusion:** We show that an increased macrophage infiltration into adipose tissue occurs already in obese children. In addition, we present evidence that BAT is present in subcutaneous adipose tissue of lean children.

## PS 049 Secreted proteins and organ cross-talk

### 679

#### Adipokine profiles discriminate different obesity mouse models

S. Hartwig<sup>1</sup>, B. Knebel<sup>1</sup>, H.-D. Dicken<sup>2</sup>, J. Kotzka<sup>1</sup>, S. Lehr<sup>1</sup>;

<sup>1</sup>Institute for Clinical Biochemistry and Pathobiochemistry, <sup>2</sup>National Diabetes Information Center, German Diabetes Center, Düsseldorf, Germany.

**Background and aims:** The group of adipokines comprises hundreds of biological active proteins and peptides released from adipose tissue. Alteration of those complex protein signatures are suggested to play a crucial role within pathophysiology of multifactorial, metabolic diseases. Our previous proteomic profiling studies uncovered the complex nature of the adipokine and identified several novel adipokines. To make the data available, we build up a database. Next to this, we started advanced investigation of adipokine profiles of lean and obese mouse models using a combined transcriptome and proteome analysis approach in order to identify potential biomarker candidates.

**Material and methods:** Explants from visceral fat depots derived from different mouse models, i.e. C57Bl6, dbdb and alb-SREBP-1a, were investigated. The dbdb mice represent the obese model induced by hyperphagia, whereas alb-SREBP-1a increases adipose tissue by elevated *de novo* lipid synthesis. At RNA level the different transcriptomes were investigated by Affymetrix-Array and RT-PCR and the secretome profiling at protein level were done by Cytokine-Arrays and the 2D-DIGE technology coupled with mass spectrometry.

**Results:** We have generated a tissue specific secretome database, labeled *Diabetesityprot* ([www.diabetesityprot.org](http://www.diabetesityprot.org)). With its functionality it is now possible to perform a target or a global search. Based on our published adipokine list of 250 annotated proteins we could confirm the expression of adipokine pattern by transcriptome data ( $>10.000$  transcripts) achieved from viscera fat depots of our mouse models. 94% of proposed adipokines were found to be present in the investigated models. Comparison analysis of the lean control with the obese mouse models identified 24 adipokines to be differentially expressed. Alpha-1-Antitrypsin (SERPINA1) a novel adipokine described to be decreased in plasma of type 2-diabetics solely could be detected in the non obese mice. Analyzing adipose tissue secretomes with cytokine arrays revealed a dramatically difference between the both obese models, e.g. IL6 was significantly elevated in dbdb mice whereas the level in alb-SREBP-1a mice was significantly low. In addition to that, 2D-DIGE secretome pattern analysis allows discriminating the two models of obesity in 50 protein spots.

**Conclusion:** The parallel utilization of transcriptome and proteome approaches and combination with systematic analysis of databases identifies differences in secretion profiles of mice displaying different characteristics of obesity. This may pave the road to identify and validate novel biomarker candidates being involved in the pathogenesis of insulin resistance and type-2-diabetes.

## 680

#### Vaspin is related to eating behaviour in humans

J. Breitfeld<sup>1</sup>, D. Schleinitz<sup>1</sup>, A. Tönjes<sup>1,2</sup>, B. Enigk<sup>1</sup>, M. Prellberg<sup>1</sup>, M. Stumvoll<sup>1</sup>, M. Blüher<sup>1</sup>, P. Kovacs<sup>1</sup>, Y. Böttcher<sup>2</sup>;

<sup>1</sup>Department of Medicine, University of Leipzig, <sup>2</sup>IFB Adiposity Diseases, University of Leipzig, Germany.

**Background and aims:** Vaspin (visceral adipose tissue derived serin protease inhibitor, serpinA12) is a novel adipokine, which might provide a link between insulin resistance and obesity. Vaspin follows a meal-related diurnal variation in humans and its intracerebroventricular administration in db/db mice leads to reduced food intake and sustained significant improvement in glucose concentrations, thus suggesting a role in the regulation of food intake. Here, we investigated the relationship between vaspin and eating behaviour in humans by analysing the correlations between serum vaspin levels, eating behaviour and genetic variants in the vaspin gene.

**Materials and methods:** We assessed eating behaviour by using the three-factor eating questionnaire in 618 Sorbian subjects from Germany. Serum vaspin levels were measured by ELISA. We performed correlation analyses

between eating behaviour and serum vaspin as well as association analyses with genetic variants.

**Results:** We found positive correlations between serum vaspin concentrations and factors measuring restraint ( $P=0.006$ ), disinhibition ( $P=0.004$ ) and hunger ( $P=0.01$ ). These data have been independently supported by genetic association analyses in which two single nucleotide polymorphisms (rs2236242, rs3736803) were significantly associated with both serum vaspin ( $P<10^{-8}$ ) and measures of eating behaviour ( $P<0.05$ ; all in regression analyses adjusted for age, sex and BMI in additive mode of inheritance).

**Conclusion:** Correlations between serum vaspin and eating behaviour along with associations of genetic variants with both vaspin concentrations and with eating behaviour suggest the role of vaspin in the regulation of eating behaviour.

Supported by: DFG KO3880/1-2

## 681

### The role of visfatin and retinol binding protein 4 in glycaemic and insulinaemic status of IGT

I. Khan<sup>1</sup>, F. Kabir<sup>2,3</sup>, F.A. Jahan<sup>2</sup>, M.O. Faruque<sup>2</sup>, Z. Hassan<sup>1</sup>, L. Ali<sup>2,4</sup>;

<sup>1</sup>Dept of Physiology and Molecular Biology, BIRDEM, <sup>2</sup>Dept of Biochemistry and Cell Biology, BIRDEM, <sup>3</sup>Dept of Biochemistry, Green Life Medical College, <sup>4</sup>Dept of Biochemistry and Cell Biology, Bangladesh Institute of Health Sciences, Dhaka, Bangladesh.

**Background and aims:** Visfatin and retinol binding protein 4 (RBP4) have been reported to be associated with insulin resistance in T2DM subjects. However, their causal association in the development of T2DM is still unclear. Since, impaired glucose tolerance (IGT) is one of the intermediate stages of natural history of T2DM, we studied these adipocytokines in relation with glycemic and insulinemic status of a group of IGT.

**Subjects and methods:** Fifty eight IGT subjects were recruited purposively and 52 age-, BMI- and body fat mass (BFM)- matched healthy subjects served as control [Control vs IGT: Age (Yrs),  $42\pm5$  vs  $42\pm6$ ; BMI ( $\text{Kg}/\text{m}^2$ ),  $24.5\pm4.1$  vs  $25.5\pm4.7$ ; BFM (%),  $28.2\pm6.2$  vs  $29.3\pm7.5$ ]. Anthropometrics and total body fat mass were determined following standard procedure. Glucose and lipids were measured by standard biochemical method. Insulin, visfatin and RBP4 were estimated by using enzyme linked immunosorbent assay (ELISA). Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were determined by homeostasis model assessment (HOMA) using HOMA-Sigma software. Data were analyzed by appropriate univariate as well as multivariate tools.

**Results:** Waist to hip ratio (WHR), blood pressures and lipids did not show statistical difference between groups except triglyceride (TG) which was found significantly higher in the IGT group compared to Controls ( $p<0.001$ ). Fasting insulin ( $\mu\text{U}/\text{ml}$ ) in the IGT group was significantly higher compared to the Controls ( $p=0.001$ ). HOMA%S (Control:  $81.9\pm18$ , IGT:  $66.5\pm18$ ) was significantly lower in the IGT compared to the Controls ( $p<0.001$  respectively). However, HOMA%B did not show any difference between the groups. Mean fasting visfatin ( $\text{ng}/\text{ml}$ ) was (Control:  $2.8\pm2.5$ , IGT:  $5.2\pm4.3$ ) was significantly higher compared to the Controls ( $p=0.004$ ). Fasting RBP4 ( $\mu\text{g}/\text{ml}$ ) (Control:  $30.4\pm6.1$ , IGT:  $36.4\pm8.6$ ) was also significantly higher in IGT group compared to the Controls ( $p<0.001$ ). Even after adjusting the effect of age, BMI, WHR, BFM and TG in binary logistic regression both Visfatin ( $p=0.017$ ) and RBP4 ( $p=0.004$ ) showed significant association with IGT group. In both bivariate correlation analysis and multiple linear regression analyses (after adjusting the effect of age, BMI, WHR, BFM and TG) both Visfatin and RBP4 showed significant positive association with postprandial glucose and fasting insulin, and significant negative association with HOMA%S (for all  $p<0.05$ ). Moreover, visfatin and RBP4 found to be correlated with each other in bivariate ( $p=0.027$ ) and multivariate (age, BMI, WHR and BFM adjusted) ( $p=0.036$ ).

**Conclusion:** Visfatin and RBP4 are associated with impaired postprandial glucose disposal in IGT subject and their role in insulin resistance may be interlinked.

Supported by: Diabetic Association of Bangladesh, Bangladesh

## 682

### Expression of the complement system is upregulated in subcutaneous adipocytes from non-obese hypertriglyceridaemic subjects and is associated with local insulin resistance

M.M.J. van Greevenbroek<sup>1</sup>, S. Ghosh<sup>2</sup>, C.J.H. van der Kallen<sup>1</sup>, M.C.G. Brouwers<sup>1</sup>, C.G. Schalkwijk<sup>1</sup>, C.D.A. Stehouwer<sup>1</sup>;

<sup>1</sup>CARIM school for cardiovascular diseases / Department of Internal Medicine, Maastricht University, Maastricht, Netherlands, <sup>2</sup>North Carolina Central University, Durham, USA.

**Background and aims:** Dysfunctional adipose tissue plays an important role in the etiology of a large number of cardiometabolic disorders (such as dyslipidaemia, the metabolic syndrome and type 2 diabetes), independent of the presence of overt obesity. However, the molecular mechanisms underlying adipocyte dysfunction, especially in metabolically stressed non-obese subjects, are incompletely understood. A metabolic disorder involving adipose tissue dysfunction in the absence of pronounced obesity is familial combined hyperlipidaemia (FCHL). In the current investigations, FCHL served as a model of familial insulin resistance in the presence of dyslipidaemia. Our aim was to identify differentially expressed pathways in human adipocytes isolated from healthy controls and dyslipidemic patients, using transcriptome analyses.

**Materials and methods:** Whole genome expression profiling on Affymetrix GeneChip<sup>®</sup> Human Genome U133 Plus 2.0 arrays was conducted on RNA of isolated subcutaneous adipocyte fractions (free of stromal vascular cells) from 13 marginally overweight FCHL subjects (body mass index (BMI)  $26.6 \pm 2.4 \text{ kg}/\text{m}^2$ ) and 8 unrelated controls of comparable body sizes ( $25.2 \pm 3.8 \text{ kg}/\text{m}^2$ ,  $p=0.298$ ).

**Results:** Gene set enrichment and co-expression network analysis identified the complement system and its regulators as one of the top upregulated pathways in FCHL ( $\text{FDR}<1 \times 10^{-30}$ ). Complement gene expression was associated with plasma triglycerides and waist circumference, and this association was independent of age and sex. Moreover, expression of several complement genes of the classical, the alternative and the terminal pathway was inversely associated with the expression of IRS-1 in the adipocytes (Pearson's correlation coefficient ( $r$ ) ranging from  $-0.45$  to  $-0.76$ ,  $p=0.05$  to  $<0.001$ ). Hypertriglyceridaemia per se was most likely not the immediate driver of complement pathway upregulation since, in a separate intervention study of 12 FCHL subjects (8 weeks treatment 40 mg/day atorvastatin monotherapy), a 40% reduction in triglycerides did not affect complement expression in adipocytes isolated before and after the intervention.

**Conclusion:** These findings point to an upregulation of complement related transcriptome in subcutaneous adipocytes under metabolically stressed conditions, even in the absence of overt obesity. Such upregulation may subsequently influence downstream processes including macrophage infiltration into adipose tissue, insulin resistance, adipocyte apoptosis and cardiovascular risk.

## 683

### Fetuin-A influences vascular cell growth and production of proinflammatory and angiogenic proteins by human perivascular fat cells

D.I. Siegel-Axel<sup>1</sup>, K. Rittig<sup>1</sup>, N. Stefan<sup>1</sup>, J.H. Dolderer<sup>2</sup>, H.-E. Schaller<sup>2</sup>, U.A. Stock<sup>3</sup>, H.-U. Haering<sup>1,4</sup>;

<sup>1</sup>Department of Internal Medicine, Medical Clinic IV, Division of Endocrinology, Diabetology, Angiology, Nephrology and Clinical Chemistry, University Tuebingen, <sup>2</sup>Department of Plastic, Hand and Reconstructive Surgery, BG-Trauma-Center, University Tuebingen, <sup>3</sup>Department of Thoracic, Cardiac and Vascular Surgery, University Tuebingen, <sup>4</sup>Institute for Diabetes Research and Metabolic Diseases Helmholtz Center Munich University of Tuebingen (Paul Langerhans Institute Tuebingen), Partner in the German Center for Diabetes Research, Germany.

**Background and aims:** Fetuin-A or alpha-2-HS-glycoprotein, a member of the superfamily of cystatins, is produced by the fatty liver. Fetuin-A is involved in insulin resistance and diabetes mellitus, in bone metabolism, central nervous system, neurodegenerative diseases but maybe also in the cardiovascular system. Fetuin-A binds insulin receptors in muscle and fat cells, inhibits adiponectin expression and increases TNF $\alpha$  expression in human subcutaneous adipocytes. Recently, we found that perivascular fat cells (PVFC) have a higher angiogenic potential than other fat cell types. Aim of this study was to examine if fetuin-A influences the expression and secretion



of pro-inflammatory and angiogenic proteins by PVFC, and the growth of vascular smooth muscle cells (SMC) and endothelial cells (EC).

**Materials and methods:** PVFC, EC and SMC were isolated from human specimens of arm arteries from patients of the BG Trauma Center and the Department of Thoracic, Cardiac and Vascular Surgery. These cells were characterized by FACS methods and cocultured in transwell systems. In one half of the cultures human fetuin-A was added in doses between 300–600 µg/ml. After 6, 24, 48, and 72 h, supernatants were collected and cells were lysed for mRNA extraction. For the quantification of the release of pro-inflammatory and angiogenic proteins, the luminex technology was used and for mRNA expression realtime PCR. Furthermore, a specific ELISA against HGF in mono- and cocultures was performed. Finally, BrDU-ELISA and WST-assays were used to analyse growth and viability of EC and SMC under the influence of fetuin-A.

**Results:** After 6 h, but predominantly after 24–72 h, PVFC expressed and secreted the angiogenic factors PAI-1, IL-6, IL-8, bFGF, PDGF-BB, MCP-1, VEGF, PlGF and HGF. This was stimulated by the cocultivation with vascular cells. IL-8 and PlGF increased even up to 10-fold compared to monocultures. Fetuin-A inhibited the IL-8 production of PVFC, whereas the secretion of VEGF was stimulated. Furthermore, the release of HGF, which we could identify recently as an important angiogenic factor of perivascular fat released in serum of patients, was inhibited by fetuin-A. Finally, SMC-growth, and moderately also EC-growth, was inhibited by fetuin-A.

**Conclusion:** In human PVFC and vascular cells fetuin-A influences the expression and secretion of several proinflammatory and angiogenic factors and the growth of vascular cells. This extends previous studies of our group showing that fetuin-A induces mRNA expression of IL-6, IL1β, MCP-1 and TNF-α in subcutaneous adipocytes. Thus, fetuin-A maybe regulates interactions of angiogenic and proinflammatory factors of fat cells and vascular cells. This might be important for neovascularization of advanced atherosclerotic plaques.

*Supported by: BMBF to the German Center for Diabetes Research (DZD) and REGINA 01KQ0902F*

immune and inflammatory responses, chemotaxis, cell growth and apoptosis were significantly over-represented in CAF rats. Real-time PCR confirmed changes for most statistically relevant genes. Interestingly, genes involved in inflammatory response were down-regulated in islets of CAF rats. Metabolites involved in glycolysis, lipidic and aminoacids metabolism were significantly changed in CM from CAF rats. Decreased glucose consumption and lactate secretion and increased glutamine consumption and α-ketoglutarate, malate and oleic acid secretion in CM from CAF rats suggest decreased glycolysis and enhanced TCA anaplerosis. Interestingly, nicotinamide was significantly increased in CM from CAF rats, which may have a protective role on diet induced islet inflammation. The generated functional networks show that possible interrelations between genes and metabolites obtained in this study are involved in inflammatory response, cell-cell signalling, cell death and proliferation and lipid metabolism.

**Conclusion:** Preliminary meta-analysis of combined networks shows interesting interplay between genes and metabolites, supporting the hypothesis of a cross-talk between pWAT and beta-cells.

*Supported by: European Commission (FP7-MC-IAPP-218130)*

## 684

### Integration of transcriptomics and metabolomics data to unravel the cross-talk between adipose tissue and beta cells during evolution of obesity

R. Malpique<sup>1,2</sup>, H. Figueiredo<sup>1,2</sup>, M. Vinaixa<sup>3,2</sup>, S. Rebuffat<sup>1,2</sup>, S.G. Kalko<sup>4</sup>, X. Correig<sup>3,2</sup>, R. Gomis<sup>1,2</sup>

<sup>1</sup>Diabetes and Obesity, IDIBAPS - Hospital Clínic, Barcelona, <sup>2</sup>CIBERDEM, Barcelona, <sup>3</sup>Metabolomics Platform, IISPV - Universitat Rovira i Virgili, Tarragona, <sup>4</sup>Bioinformatics Unit, IDIBAPS - Hospital Clínic, Barcelona, Spain.

**Background and aims:** During evolution of obesity, an increase in beta cell mass occurs to cope with the raise in insulin demand, which is essential to avoid the onset of hyperglycemia. Changes in the adipokine secretion profile of visceral-pancreatic white adipose tissue (pWAT) due to diet-induced obesity have been shown to increase beta cell replication, suggesting that a cross-talk between pWAT and beta cells may play a role in regulating beta cell plasticity. We aimed to elucidate the molecular mechanisms involved in this cross-talk by investigating changes on pWAT gene expression and metabolite secretion due to diet-induced obesity and correlate them with changes on the transcription profile of beta-cells.

**Materials and methods:** Male Wistar rats were exposed to a 30 days high caloric cafeteria (CAF) diet or standard chow diet (n=6/group). The animals were sacrificed and pWAT and pancreatic islets were collected. Conditioned medium (CM) was obtained from pWAT by culturing in serum-free medium for 24 hours. A comparative CM analysis was performed using multiplatform untargeted metabolomics (1H-NMR, GC/MS and HPLC/MS). Transcription profiles of both tissues were analyzed using Affymetrix Rat 230.2 whole genome arrays. Statistical analyses of metabolomics and transcriptomics data were performed using Mann-Witney (p<0.05) and RankProd methods (FDR 0.01), respectively. Analysis of functional over-representation was performed according to the GO-BP database. The Ingenuity Systems Pathway Analysis software was used for cross-platform integration.

**Results:** Microarray analysis revealed the up-regulation of 45 genes in pWAT of CAF rats, whereas down-regulation of 108 genes and up-regulation of 31 genes were found for islets of CAF rats. The functional terms related to the positive regulation of immune and inflammatory responses, hormone stimulus and fatty acid metabolism were significantly over-represented in pWAT of CAF rats. In islets, functional terms corresponding to the regulation of

## PS 050 Metabolic regulation in the brain

685

### Free fatty acid receptors (FFAR1) mediate effects of conjugated linoleic acid in brain

T. Sartorius<sup>1,2</sup>, A. Peter<sup>1</sup>, C. Weigert<sup>1</sup>, A.M. Hennige<sup>1</sup>, E. Schleicher<sup>1</sup>, E. Kostenis<sup>3</sup>, H.-U. Haering<sup>1</sup>, S. Ullrich<sup>1</sup>;

<sup>1</sup>Division of Endocrinology, Diabetology, Vascular Disease, Nephrology and Clinical Chemistry, University of Tuebingen, <sup>2</sup>Institute for Diabetes Research and Metabolic Diseases (IDM) of the Helmholtz Center Munich at the University of Tuebingen, <sup>3</sup>University of Bonn, Germany.

**Background and aims:** Conjugated linoleic acids (CLAs) are attractive nutritional additives since they influence body fat distribution and increase muscle mass. However, the intake of CLAs results in adverse effects such as worsening of peripheral insulin resistance and increasing the risk of developing diabetes. We have previously found that CLAs, both the cis-9, trans-11-, and the trans-10, cis-12-CLA isomer, act through the activation of the free fatty acid receptor 1 (FFAR1 former GPR40) in insulin secreting cells. FFAR1 is also expressed in brain and regulates certain neurological functions. The aim of the present study was to analyse whether orally administered CLAs affect brain function and to decipher the role of FFAR1.

**Materials and methods:** FFAR1 null mice and their WT littermates at the age of 6–10 weeks were orally administered by gavage with CLAs or sunflower oil (control) for 30 days. The impact on glucose homeostasis was evaluated by intraperitoneal GTT and brain fatty acid composition by gas chromatography. Radiotelemetry recordings were performed to assess brain activity and locomotor behaviour after an acute intracerebroventricular (icv) application of the CLA isomers to achieve a final concentration of 100 µmol/l.

**Results:** In brain of WT mice, the concentration of CLA significantly increased 2.3-fold after CLAs feeding ( $P < 0.005$ ,  $n = 4$ ). The respective concentrations in FFAR1 null mice were not significantly different to WT. However, a prominent role of the FFAR1 in brain function could be detected after an acute administration of the two CLA isomers. Acutely administered 10/12 CLA isomer in the cerebrospinal fluid of WT mice stimulated brain activity in the low frequency band (delta) more efficiently than the 9/11 CLA isomer (delta: by  $27.1 \pm 7.1\%$  (9/11 CLA) vs.  $51.9 \pm 8.3\%$  (10/12 CLA);  $P < 0.05$ ;  $n = 4$ /group). In FFAR1 null mice no significant difference was found between the two CLA isomers (delta:  $23.7 \pm 4.9\%$  (9/11 CLA) vs.  $24.7 \pm 5.4\%$  (10/12 CLA);  $n = 5$ /group). Interestingly, locomotor activity was reduced after acute icv administration of 9/11 CLA isomer in WT but not in FFAR1 null mice (WT:  $52.5 \pm 5.6\%$  vs. vehicle  $100 \pm 8.4\%$ ;  $P < 0.001$ ). In addition, insulin resistance provoked by chronic CLAs feeding was also not dependent on FFAR1 expression. **Conclusion:** Orally ingested CLAs accumulate in brain tissue and affect brain activity and locomotion. These effects are at least partially mediated by FFAR1. Our results suggest that orally ingested CLAs not only exert adverse effects in the periphery but also in the brain.

Supported by: BMBF (DZD e.V.)

686

### Regulation of AMP-activated protein kinase alpha 2 expression by a fasting/ghrelin-induced transcription factor, AF5q31, in the hypothalamic neurons

T. Komori<sup>1</sup>, A. Doi<sup>2</sup>, T. Nosaka<sup>3</sup>, H. Furuta<sup>2</sup>, T. Akamizu<sup>2</sup>, T. Kitamura<sup>4</sup>, E. Senba<sup>1</sup>, Y. Morikawa<sup>1</sup>;

<sup>1</sup>Anatomy and Neurobiology, Wakayama Medical University, <sup>2</sup>The First Department of Medicine, Wakayama Medical University, <sup>3</sup>Department of Microbiology, Mie University Graduate School of Medicine, <sup>4</sup>Division of Cellular Therapy, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Japan.

**Background and aims:** In the hypothalamus, fasting induces a member of the AF4 family of transcription factors, AF5q31, originally identified as a fusion partner of the MLL (mixed-lineage leukemia) gene in infant acute lymphoblastic leukemia. However, the roles of AF5q31 in the hypothalamus remain unclear. In the present study, we reported a novel functional role of AF5q31 in the hypothalamic neurons.

**Materials and methods:** The effects of fasting and ghrelin on the expression of AF5q31 were examined in mice. We used a hypothalamic neuronal cell

line, GT1-7 cells, to investigate the direct effect of ghrelin on the expression of AF5q31 and the roles of AF5q31 on the expression of various AMP-activated protein kinase (AMPK) subunit isoforms.

**Results:** The expression of AF5q31 was increased in the neurons of the arcuate nucleus and ventromedial hypothalamic nucleus (VMH) of fasted mice compared to those of mice fed ad libitum. AF5q31 expression was exclusively observed in growth hormone secretagogue-receptor (GHS-R)-expressing neurons during fasting. The intraperitoneal injection of ghrelin induced the expression of AF5q31 in the GHS-R-positive neurons of the arcuate nucleus and VMH. To examine the direct effect of ghrelin on the expression of AF5q31, we used the hypothalamic cell line GT1-7, in which GHS-R is expressed. In GT1-7 cells, ghrelin markedly induced the expression of AF5q31 in a time- and dose-dependent manner, suggesting that ghrelin directly induce the expression of AF5q31 in the hypothalamus during fasting. Following the induction of AF5q31 expression by ghrelin, the expression of AMPKα and phosphorylation of acetyl CoA carboxylase α (ACCα), a downstream target of AMPK, were increased in GT1-7 cells. These results raise the possibility that AF5q31 regulates the AMPK signaling pathway through the regulation of AMPKα expression. To test this possibility, we transfected full-length AF5q31 in GT1-7 cells. The overexpression of AF5q31 in GT1-7 cells specifically induced the expression of the AMPKα2 subunit, but failed to induce other AMPK subunits and AMPK upstream kinases. In addition, the promoter activity of AMPKα2 gene increased by the addition of AF5q31, suggesting that AF5q31 regulates the transcription of AMPKα2 gene.

**Conclusion:** AF5q31 induced by fasting and ghrelin may modulate AMPK signaling by inducing the expression of AMPKα2 in the hypothalamus.

Supported by: KAKENHI(No.20790724, No. 23659385), Chiyoda Mutual Life Foundation

687

### Insulin detemir reduces activity in reward-related brain regions in response to visual food stimuli in type 1 diabetes: possible explanation for its weight-sparing effect?

L.W. van Golen<sup>1</sup>, D.J. Veltman<sup>2</sup>, R.G. Ijzerman<sup>1</sup>, J.B. Deijen<sup>3</sup>, M.L. Drent<sup>1</sup>, F. Barkhof<sup>4</sup>, M. Diamant<sup>1</sup>;

<sup>1</sup>Diabetes Center/ Department of Internal Medicine, <sup>2</sup>Department of Psychiatry, <sup>3</sup>Department of Clinical Neuropsychology, <sup>4</sup>Department of Radiology, VU University Medical Center, Amsterdam, Netherlands.

**Background and aims:** Insulin detemir therapy is associated with less weight gain than other insulin therapies like NPH insulin, but the underlying mechanisms have not been elucidated. Functional magnetic resonance imaging (fMRI) studies have shown an increased activity in reward-related brain regions when obese subjects are confronted with visual food stimuli. Since insulin enters the brain and acts as a satiety hormone, we hypothesised that treatment with insulin detemir would result in decreased activity in these reward-related brain regions in patients with type 1 diabetes (T1DM) when viewing food pictures compared to treatment with NPH insulin.

**Materials and methods:** Thirty-two T1DM males (mean ± SD; age  $36.3 \pm 9.4$  years, body weight  $83.9 \pm 13.8$  kg, BMI  $25.3 \pm 3.2$  kg·m<sup>-2</sup>, diabetes duration  $13.0 \pm 8.6$  years, HbA1c  $7.3 \pm 0.6\%$ ) were included in a randomised cross-over study. Patients were treated with a basal bolus regimen for 2 periods of 12 weeks, starting with either insulin detemir or NPH insulin, in combination with insulin aspart. After each treatment period, all patients had an fMRI early in the morning in the fasted state. During the scan, pictures were shown of either food or non-food items and patients had to perform a memory encoding task. Hunger ratings before and after scanning were scored on a Likert scale. Eating behaviour was scored using the DEBQ. Imaging data were analysed in SPM8 and peak level maps were obtained with a threshold at  $< 0.05$ .

**Results:** After 12 weeks, daily insulin doses and HbA1c were similar in both treatment groups. Insulin detemir decreased body weight by 0.8 kg, whereas NPH insulin increased weight by 0.5 kg (between-group difference  $p = 0.02$ ). During fMRI, mean glucose levels were  $8.8 \pm 3.6$  mmol/l in the detemir group versus  $10.4 \pm 4.0$  mmol/l in the NPH insulin group ( $p = 0.046$ ); no differences between groups were observed in hunger ratings or eating behaviour. Patients treated with NPH insulin showed increased brain activity in the bilateral insula when watching food pictures versus non-food pictures compared to patients treated with insulin detemir ( $p < 0.04$  FWE); these differences between groups were not affected by adjustment for glucose levels.

**Conclusion:** Treatment with insulin detemir resulted in reduced activation in reward-related brain regions in response to visual food stimuli compared to NPH insulin treatment in T1DM patients, which was paralleled by a decrease in body weight after 12 weeks of treatment. These results lend support to the

hypothesis that insulin detemir results in less weight gain by enhancing satiety signals on the brain.

*Clinical Trial Registration Number:* NCT00626080

*Supported by:* Novo Nordisk A/S IIT grant

## 688

### The prevalence of impaired glucose regulation in patients with sleep disorder and its relationship with altered HPA and HPT axis activity

J. Li<sup>1</sup>, Y. Yu<sup>1</sup>, X. Sun<sup>2</sup>;

<sup>1</sup>Endocrinology and Metabolism, <sup>2</sup>Mental Health Center, West China Hospital, West China Medical School, Sichuan University, Chengdu, China.

**Background and aims:** Sleep disorder such as early awakenings and difficulties with initiation of sleep is associated with adverse effect on glucose regulation, but few studies have examined its association with prevention of type 2 diabetes. This study was designed to evaluate the fasting glucose, tolerance glucose and the concentration of plasma insulin, assess the function of both hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-adrenal (HPA) axis and to investigate the relationship of altered hypothalamic function with sleep disorder.

**Materials and methods:** From January 2010 to December 2011, 501 patients including 324 female (64.7%) and 177 male (35.32%) diagnosed with sleep disorder participated in this cross-sectional study in psychiatric outpatient department. The data of 75g glucose tolerance tests, insulin releasing test, morning (8:00) serum cortisol, TSH TT3 TT4 FT3 FT4, were collected as well as BMI and WHR to assess prevalence of IGR and function of HPA and HPT axis.

**Results:** 301 patients were diagnosed with anxiety disorder (78%) and 200 patients were diagnosed with depression (22%). The crude prevalence rates were 15.0% for diabetes, 11.6% for IGT, 15.8% for IFG, and 11.6% for IGR (IGT+IFG). The total prevalence of impaired glucose regulation in sleep disorder was 48.8%. These rates are significantly higher than the prevalence found in the general adult population. The mean cortisol level was  $463.5 \pm 178.8$  nmol/L, and the cortisol concentration at 8:00 a.m. was significantly associated with higher IGR prevalence and insulin resistance. The TSH ranged from 0.005–12.8 mU/L, median 2.76 mU/L, TSH values above 2 mU/L accounted for over 58%. FT4 ranged from 8.16–47.38 pmol/L, median 15.52 pmol/L. An increased prevalence of IGR in sleep disorders relative to the general population is highly suggested by the literature.

**Conclusion:** These results partially confirm that high level of cortisol and the increased activity of the HPT axis are associated with impaired glucose regulation. Therefore, as a pathophysiological event, abnormal activity of hypothalamic function of the sleep disorder patients could be viewed as a potential risk factor for increasing incidence of diabetes.

## 689

### MC4R genetic variants associated with fat consumption and lower visceral fat accumulation in humans

M. Gorska<sup>1</sup>, E. Adamska<sup>1,2</sup>, A. Citko<sup>1</sup>, N. Wawrusiewicz-Kurylonek<sup>1</sup>, J. Gościk<sup>3</sup>, M. Waszczeniuk<sup>2</sup>, J. Pliszka<sup>4</sup>, J. Wilk<sup>1</sup>, A. Krętowski<sup>1</sup>;

<sup>1</sup>Department of Endocrinology, Diabetology and Internal Medicine,

<sup>2</sup>Department of Dietetics and Clinical Nutrition, <sup>3</sup>Centre for Experimental

Medicine, <sup>4</sup>Department of Gynecology, Medical University, Białystok, Poland.

**Background and aims:** It is generally believed that Interaction between life-style and genetic factors play a role in the obesity development. More than 40 novel loci associated with adiposity have been recently identified. Melanocortin-4-receptor (MC4R) gene is the most common form of monogenic obesity; however, the functional effects of polymorphic variants of MC4R variants in general populations remain uncertain. The aim of our study was to analyze whether common genetic variants near the MC4R gene associated with obesity in GWAS influence diet preferences, physical activity, visceral fat accumulation and energy expenditure.

**Materials and methods:** We genotyped previously identified MC4R SNPs: rs17782313, rs633265, rs1350341, rs12970134 in 449 overweight/obese patients and 227 healthy volunteers with normal weight (289 men and 387 women), who underwent anthropometry (BMI, WHR) and body composition analysis: percent body fat, visceral (VAT) and subcutaneous abdominal adipose tissue (SAT) by multi-frequency bio-impedance method. In a subgroup of 358 subjects the 3-day diary analysis was performed and intake of

calories, carbohydrates, lipids and protein was analyzed. Moreover, in randomly selected 27 subjects carbohydrate and lipid oxidation was evaluated with indirect calorimetry during two consecutive: high-fat and high-carb meal tests. Measured genotype analysis (MGA) was used to test association between SNPs and phenotypes.

**Results:** We found the significant associations of visceral fat content and VAT/SAT ratio with the distribution of genotypes of the MC4R studied SNPs ( $p=0.003$ ). Subjects with GG genotype (rs1350341), who had the lowest visceral fat accumulation presented 2 fold higher carbohydrate oxidation rate after high-carb meal ( $p=0.043$ ) and surprisingly the highest fat consumption in 3-day diet analysis ( $p=0.015$ ).

**Conclusion:** We believe that our study may help to understand the pathways that control body mass and fat deposition in humans and provide personalized treatments and prevention strategies to fight against obesity.

*Supported by:* MNiSW 4774/B/P01/2009/37



## PS 051 Cardiovascular complications

690

**Weight gain within the first year after new onset diabetes is associated with risk of cardiovascular mortality: a cohort of 8,326 primary care patients**

J. Bodegard<sup>1</sup>, J. Sundström<sup>2</sup>, B. Svennblad<sup>3</sup>, C. Östgren<sup>4</sup>, P.M. Nilsson<sup>5</sup>, G. Johansson<sup>2</sup>;

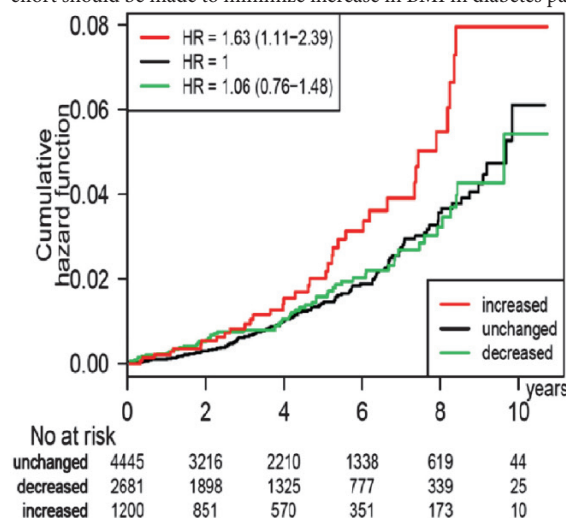
<sup>1</sup>Medical Department, AstraZeneca, Oslo, Norway, <sup>2</sup>Uppsala University, Sweden, <sup>3</sup>Uppsala Clinical Research Center, Sweden, <sup>4</sup>Linköping University, Sweden, <sup>5</sup>Lund University, Sweden.

**Background and aims:** Increased body mass index (BMI) in diabetes patients has been suggested to be associated with higher coronary heart disease risk. We explored the association between BMI changes within the first year of new onset diabetes (NOD) and the risk of cardiovascular disease (CVD) mortality.

**Materials and methods:** A cohort of 38,960 diabetes patients with NOD, no history of previous CVD or cancer, and available BMI measurements was identified at 84 primary care centers in Sweden (1999–2009). Of these, 8,326 patients had their BMI measured at least twice, at the time of NOD and within one year after the NOD diagnosis. After the last BMI measurement, the patients were followed for up to 10 years. Morbidity- and mortality data was retrieved by linking these patients to National Cause of Death and Hospital Discharge registers. Patients were grouped according to one year BMI change after NOD: “Increase”  $\geq 1$  BMI unit increase; “Unchanged” = between +1 and -1 BMI unit change; “Decrease”  $\geq 1$  BMI unit decrease. CVD risks were estimated with Cox regression, adjusted for age, gender, baseline BMI, history of angina pectoris, education, marital status and glucose lowering drugs. CVD mortality includes sudden and unexpected death, death from stroke and myocardial infarction.

**Results:** Baseline mean age was 60.0 years (range 35–79) and mean BMI 30.2 kg/m<sup>2</sup> (range 16.7–58.5). After a median follow up time of 4.6 years (38,300 patient years), most patients, 53.4%, had an unchanged BMI, 32.2% a decrease, and 14.4% an increase. At baseline, patients with increasing BMI were younger, had lower HbA1c, triglycerides and systolic blood pressure, and were during the first year with NOD more frequently on insulin and sulfonylureas; less frequently on metformin and antihypertensives compared to the unchanged group. Patients in the increased BMI group had a 63% (95%CI 1.11–2.39) increased risk of CVD death compared to patients with unchanged BMI, see figure, and all cause death increased by 33% (95%CI 1.01–1.76). In a subgroup analysis of the 3,034 patients (36.4%) increasing their BMI  $>0$  in the first year with NOD; 100 (3.3%) died from CVD and every 1 unit BMI increase was associated with 12% increased risk of CVD death (95%CI 0.94–1.33).

**Conclusion:** Increase in body mass index during the first year after new onset diabetes is associated with increased long term risk of death due to cardiovascular disease. The study also suggests that the level of BMI increase is associated with an elevated risk of dying from cardiovascular disease and every effort should be made to minimize increase in BMI in diabetes patients.



Clinical Trial Registration Number: NCT01121315

Supported by: AstraZeneca

691

**Endothelial dysfunction, endothelin-1 and inflammatory cytokines in patients with diabetic cardiomyopathy**

J.B. Belchina, L.K. Sokolova, N.D. Tronko, A.S. Efimov;  
Diabetology, Endocrinology and Metabolism, Kyiv, Ukraine.

**Background and aims:** The diabetic state is also associated with endothelial dysfunction as an initial manifestation of vascular abnormalities in diabetes. We are interested in the role of endothelin-1 and inflammatory cytokines in the pathogenesis of cardiomyopathy in patients with DM type1. The aim of this study was to assess echocardiographic evidence of cardiomyopathy and its association with endothelial function, inflammatory cytokines and endothelin-1 in type 1 normotensive non-proteinuric diabetic patients

**Materials and methods:** Fifty patients with type 1 DM (mean age 30.1  $\pm$  1.8 years) and 20 healthy controls (mean age 29.9  $\pm$  1.8 years) were studied. Each patient was underwent echocardiography and assessment of brachial endothelial function. Indexes of left ventricular diastolic filling were measured by Doppler-echocardiography. Endothelial function was investigated using ultrasound assessment of flow-mediated dilatation (FMD). ET-1, IL-10, IL-6, TNF- $\alpha$  were determined by ELISA. Data are presented as mean  $\pm$  SD. The results were compared using Student's paired test.

**Results:** A diagnosis of LVDD was made when at least one of the following parameters was present in the echocardiographic study: isovolumetric relaxation time (IRT)  $>100$ ms, deceleration time (DT)  $>220$  ms or early filling rate peak/late filling rate peak ratio (E/A)  $<1$ . According to these criterions 18 (36%) of the 50 diabetic patients had evidence of diastolic dysfunction as assessed by the presence of at least two abnormal variables of mitral inflow velocity. The percent change in diameter of the brachial artery in response to reactive hyperemia was significantly impaired in patients with DM compared with control subject, and this impairment was significantly more prominent in patients with diabetic cardiomyopathy than in those without diastolic dysfunction. So, the percent change in diameter of the brachial artery in response to reactive hyperemia in patients with diabetic cardiomyopathy (8.4  $\pm$  1.5%) was significantly lower than that in healthy volunteers (17.6  $\pm$  1.3%), ( $p < 0.05$ ), and in patients with DM but without cardiomyopathy (13.6  $\pm$  0.9%), ( $p < 0.05$ ). According to our data plasma level of ET-1 was extremely higher in patients with diabetic cardiomyopathy (2.67  $\pm$  0.38 fmol/ml) than that in healthy volunteers (0.12  $\pm$  0.02 fmol/ml), ( $p < 0.05$ ), and in patients with DM but without cardiomyopathy (1.33  $\pm$  0.23 fmol/ml), ( $p < 0.05$ ). Our results showed that an anti-inflammatory pattern of cytokine production is activated in patients with DM and cardiomyopathy, so anti-inflammatory cytokine IL-10 plasma level was higher in patients with diabetic cardiomyopathy (3.42  $\pm$  0.26 ng/ml) than that in healthy volunteers (0.29  $\pm$  0.05 ng/ml), ( $p < 0.05$ ), and in patients with DM but without cardiomyopathy (1.05  $\pm$  0.09 ng/ml), ( $p < 0.05$ ). Our data show that proinflammatory cytokines IL-6, TNF- $\alpha$  were higher in patients with DM and cardiomyopathy (2.22  $\pm$  0.28 ng/ml) and (1.82  $\pm$  0.19 ng/ml) compared in patients without diabetic cardiomyopathy (1.47  $\pm$  0.16 ng/ml) and (1.32  $\pm$  0.23 ng/ml) and in control subjects (0.31  $\pm$  0.09 ng/ml) and (0.58  $\pm$  0.12 ng/ml), respectively, ( $p < 0.05$ ).

**Conclusion:** Our data suggest that 1) more than one-third of young type 1 DM patients with a normal systolic ventricular function have diabetic cardiomyopathy; 2) impairment of endothelial function was significantly more prominent in patients with diabetic cardiomyopathy; 3) inflammatory cytokines might be able to play a key role in the pathogenesis of diabetic cardiomyopathy.

692

**Visceral but not cardiac fat is related to idiopathic dilated cardiomyopathy (DCM): a magnetic resonance study**

A. Sironi, R. Sicari, M. Gaggini, E. Buzzigoli, C. Carpeggiani, A. Gastaldelli;  
CNR Institute of Clinical Physiology, Pisa, Italy.

**Background and aims:** Excess cardiac fat, especially epicardial fat (EPI), has been related to coronary atherosclerosis and cardiac dysfunction. We have previously shown that patients with dilated cardiomyopathy (DCM) have reduced cardiac uptake/oxidation of free fatty acids (FFA) and preferential carbohydrate utilization by the heart. However, the role of EPI, if any, in DCM patients remains unsettled. Therefore, the aim of our study was to evaluate the relationship between epicardial and visceral fat with cardiac dysfunction assessed magnetic resonance (MR) in patients with DCM.

**Materials and methods:** We evaluated 99 subjects by cardiac magnetic resonance (CMR): 42 DCM patients (35/7 M/F; age 56  $\pm$  12 years, mean  $\pm$  SD) with ejection fraction  $<50\%$  (33.9  $\pm$  9.8) and angiographically normal coronary

arteries, New York Heart Association class < 3 (mean  $1.7 \pm 0.8$ ); 57 gender-matched subjects (47/10 M/F; age  $46 \pm 9$  years) with normal cardiac function. Cardiac fat, ventricular volumes, dimensions and LV function were assessed in all subjects during the MR exam and in a subset of subjects ( $n=85$ ) visceral (VF) and subcutaneous (SC) abdominal fat were also assessed by MRI.

**Results:** Patients with DCM and control subjects were matched per BMI ( $26.6 \pm 0.6$  vs.  $27.6 \pm 0.4$  kg/m<sup>2</sup>) and insulin resistance (HOMA  $3.3 \pm 0.3$  vs  $3.4 \pm 0.4$  mmol/l x mU/l) all  $p=NS$ . Total cardiac fat accumulation was similar in DCM patients and control subjects ( $2534 \pm 1092$  vs  $2589 \pm 1160$  mm<sup>2</sup>;  $p=0.81$ ). Subdivision in epicardial and extra-pericardial (ie mediastinal) fat showed no difference compared with the control group ( $854 \pm 389$  vs.  $785 \pm 334$  mm<sup>2</sup>,  $p=0.3$  and  $1747 \pm 867$  vs.  $1837 \pm 1042$  mm<sup>2</sup>,  $p=0.6$ , respectively). On the other hand visceral fat was significantly higher in CMD patients than in controls ( $1.9 \pm 0.2$  vs.  $1.3 \pm 0.1$  kg,  $p<0.01$ ) while SC fat was similar ( $2.6 \pm 0.2$  vs.  $2.6 \pm 0.2$  kg  $p=0.5$ ). In the whole data set epicardial ( $r=0.22$ ), mediastinal ( $r=0.33$ ), VF ( $r=0.36$ ) and SC ( $r=0.22$ ) fat were all positively related to HOMA (all  $p<0.05$ ). Regarding the correlation with cardiac parameters, only VF was significantly associated with increased LV mass ( $r=0.37$ ,  $p=0.001$ ), LV end systolic volume ( $r=0.33$ ,  $p=0.004$ ) and reduced EF ( $r=-0.28$ ,  $p=0.016$ ). In the logistic regression model, after accounting for BMI and gender, only visceral fat was related with CMD (OR=1.001, partial  $r=0.27$ ,  $p=0.0015$ ).

**Conclusion:** In patients with DCM, cardiac and subcutaneous abdominal fat were not increased compared to control subjects matched per BMI and insulin resistance whereas visceral fat was higher and related to the parameters of cardiac dysfunction severity.

Supported by: EFSD Clinical Research Grant

## 693

### Polarisation of circulating monocyte-macrophages is imbalanced in type 2 diabetes owing to defects in the expression of scavenger receptors

G.P. Fadini, S. Vigili de Kreutzenberg, C. Agostini, A. Avogaro;  
Department of Medicine, University of Padova, Italy.

**Background and aims:** Type 2 diabetes (T2D) is associated with subclinical chronic inflammation, which contributes to the high cardiovascular risk. Monocyte-macrophage lineage cells exist in different states of activation. Classically activated (M1) cells are pro-inflammatory and predisposed to enter the diseased vessel wall, while alternatively activated (M2) cells patrol the vessels and provide anti-inflammatory signals. M1 and M2 cells have opposing functions in both atherosclerosis and adipose tissue inflammation. We aimed to determine whether an imbalance between M1 and M2 exists at various stages of the development of T2D.

**Methods:** We enrolled 42 normal glucose tolerant (NGT) subjects, 38 pre-diabetic subjects (either IFG or IGT or both) and 43 T2D patients. We quantified circulating M1 and M2 phenotypes using flow cytometry by characterizing cells in the monocyte morphological gate: M1 were defined as CD68+CCR2+ cells and M2 were defined as CX3CR1+CD163+/CD206+ cells. Cell counts were expressed as percentage of circulating monocytes and the M1/M2 balance as the ratio between M1 and M2 percentages.

**Results:** The 3 groups of study subjects were matched for age ( $60.7 \pm 0.8$  years old) and sex (67% males). T2D patients had higher BMI, waist circumference, systolic blood pressure and lower HDL cholesterol than NGT subjects. T2D patients also had a higher prevalence of atherosclerotic cardiovascular disease. In NGT subjects,  $30.8 \pm 3.0\%$  and  $20.2 \pm 2.7\%$  of circulating monocytes had M1 and M2 characteristics, respectively (M1/M2 balance  $1.5 \pm 0.4$ ). In pre-diabetic individuals, an increase in M1 ( $39.6 \pm 3.3\%$ ;  $p=0.05$  vs NGT) was paralleled by a slight increase in M2 ( $26.8 \pm 4.3\%$ ;  $p=0.18$  vs NGT) cells, which preserved the M1/M2 balance ( $1.5 \pm 0.3$ ). On the other hand, in T2D patients, M1 cells were non significantly increased ( $34.4 \pm 2.8\%$ ) while M2 cells were markedly reduced ( $5.8 \pm 1.7\%$ ;  $p<0.0001$  vs NGT) and the M1/M2 ratio was markedly unbalanced ( $5.9 \pm 1.0$ ). The M1/M2 ratio was directly correlated with BMI ( $r=0.19$ ;  $p=0.031$ ), waist ( $r=0.22$ ;  $p=0.014$ ) and HbA1c ( $r=0.31$ ;  $p<0.001$ ). The association between M1/M2 ratio and T2D was independent of BMI, waist, blood pressure, HDL and atherosclerotic disease. The M1/M2 imbalance in T2D was mainly attributable to a reduction in the expression of the scavenger receptors CD163 and CD206, which are critical for the clearance activity of monocyte-macrophage cells.

**Conclusion:** While in IFG/IGT subjects a surge in M1 cells is balanced by M2 cells, T2D patients show a profound imbalance in pro- versus anti-inflammatory monocytes compared to NGT subjects. This is mainly attributable to a defect in the expression of scavenger receptors that are functional to the anti-inflammatory activity of M2. These alterations, that are potentially important determinants of cardiovascular risk, may be directly related to hyperglycemia.

## 694

### THOC5-a novel gene associated with HDL-cholesterol

M. Keller<sup>1</sup>, A. Tönjes<sup>2</sup>, J. Dünnebeil<sup>3</sup>, D. Schleinitz<sup>2</sup>, Y. Böttcher<sup>1</sup>, J. Breitfeld<sup>2</sup>, N.W. Rayner<sup>4</sup>, A. Fischer-Rosinsky<sup>5</sup>, M. Koriath<sup>2</sup>, A. Pfeiffer<sup>6,5</sup>, K. Krohn<sup>3</sup>, L. Groop<sup>7</sup>, J. Spranger<sup>5</sup>, M. Stumvoll<sup>1,2</sup>, P. Kovacs<sup>3</sup>;

<sup>1</sup>IFB-Adiposity Diseases, University of Leipzig, Germany, <sup>2</sup>Department of Medicine, University of Leipzig, Germany, <sup>3</sup>Interdisciplinary Centre for Clinical Research, University of Leipzig, Germany, <sup>4</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, UK, <sup>5</sup>Clinic of Endocrinology, Diabetes and Nutrition, Charité-Universitätsmedizin Berlin, Germany, <sup>6</sup>Department of Clinical Nutrition, German Institute of Human Nutrition, Nuthetal, Germany, <sup>7</sup>Department of Clinical Sciences/Diabetes and Endocrinology, Lund University, Malmö, Sweden.

**Background and aims:** Although numerous genes are known to regulate serum lipid profiles, identified variants still explain only a small proportion of expected heritability. We intended to identify further variants associated with lipid phenotypes in a self-contained population of Sorbs in Germany.

**Materials and methods:** We performed a genome wide association study (GWAS) on LDL-cholesterol (LDL), HDL-cholesterol (HDL) and triglyceride levels (TG) in 839 Sorbs. All single nucleotide polymorphisms (SNPs) with a  $P<0.01$  were taken forward to a meta-analysis including an independent Swedish cohort (Diabetes Genetics Initiative [DGI];  $n \sim 3100$ ). Novel association signals with the strongest effects were taken forward to replication studies in an additional German cohort (Berlin,  $n = 2031$ ).

**Results:** In the primary GWAS in the Sorbs we identified 14 loci associated with lipid traits with a  $P<10^{-5}$ . Moreover we could confirm significant effects for 18 previously reported loci. The combined analysis of the three study cohorts ( $n(\text{HDL}) = 6041$ ,  $n(\text{LDL}) = 5995$ ,  $n(\text{TG}) = 6087$ ) for the strongest association signals from the meta-analysis showed significant effects of the THOC5 variant rs8135828 on HDL serum concentrations ( $P=1.78 \times 10^{-7}$ ; Z-Score = 5.221). Genotyping of 4 tagging SNPs in the initial study population (Sorbs) within the THOC5 revealed 3 SNPs (rs8140060, rs4823045, rs2283860) moderately associated with HDL-cholesterol (all  $P \leq 0.05$ ).

**Conclusion:** We propose THOC5 to be a novel locus associated with serum HDL levels. Functional investigations to further support the role of THOC5 in HDL metabolism are currently ongoing.

Supported by: GDA, IFB(K7-37), DFG (KFO 152), (SP716/2-1), DFG(GK1208), IFB(ADI-K5D and K7-38)

## 695

### Weight loss and improvements in cardiometabolic outcomes with extended-release phentermine/topiramate treatment in overweight/obese subjects with type 2 diabetes

A.V. Astrup<sup>1</sup>, J.-M. Oppert<sup>2</sup>, C.A. Peterson<sup>3</sup>;

<sup>1</sup>Department of Human Nutrition, LIFE, University of Copenhagen, Frederiksberg, Denmark, <sup>2</sup>Department of Nutrition, University Pierre-et-Marie Curie, Paris, France, <sup>3</sup>Vivus, Inc., Mountain View, USA.

**Background and aims:** Treatment targets for overweight and obese patients with type 2 diabetes mellitus (T2DM) include weight loss, reduction of glycosylated haemoglobin (HbA1c) to <6.5%, and reduction of systolic blood pressure (SBP) to <130 mg/dL. Previously, CONQUER, a 56-week, randomised study of overweight/obese subjects with  $\geq 2$  weight-related comorbidities, demonstrated significant weight loss with phentermine plus extended-release topiramate (PHEN/TPM ER) compared with placebo. This post-hoc analysis assessed changes in weight, HbA1c, and SBP, and the percentage of subjects who achieved the composite goal of weight loss >10%, HbA1c <6.5%, and SBP <130 mm Hg in subjects with T2DM at baseline.

**Materials and methods:** Subjects received lifestyle intervention plus placebo, PHEN 7.5 mg/TPM ER 46 mg (7.5/46), or PHEN 15 mg/TPM ER 92 mg (15/92) and were managed to T2DM standards of care. Least-squares (LS) mean percent weight loss and changes in HbA1c and SBP were assessed at week 56 in subjects with T2DM at baseline. The proportions of subjects with T2DM at baseline who achieved the composite goal at week 56 were also evaluated.

**Results:** In total, 388 subjects had T2DM at baseline: lifestyle intervention plus placebo ( $n=157$ ), 7.5/46 ( $n=67$ ), or 15/92 ( $n=164$ ). Percent weight loss and reductions in HbA1c were significant vs placebo for all comparisons; changes in SBP were not significant vs placebo (Table). In total, 62 subjects (6 [3.8%], 9 [13.4%], and 47 [28.7%] in the placebo, 7.5/46 and 15/92 groups, respectively) achieved the composite goal. In subjects with T2DM at baseline,

there were 6 reports of hypoglycaemia: 4 in the placebo group (1 severe; 3 mild) and 1 in each of the 7.5/46 and 15/92 groups (both mild). Discontinuation due to adverse events was 8%, 9%, and 19% for placebo, 7.5/46, and 15/92, respectively, with the most common adverse events being constipation, upper respiratory tract infection, and paraesthesia.

**Conclusion:** These findings show that in obese subjects with T2DM, weight loss induced by PHEN/TPM ER is associated with improvements in HbA1c and SBP. This indicates that PHEN/TPM ER treatment may facilitate the achievement of composite treatment goals that are recommended for the management of T2DM.

Table. LS mean change in endpoints at week 56 in subjects with T2DM at baseline (ITT-LOCF)

	Placebo	7.5/46	15/92
Weight loss, %	1.9	6.8*	8.8*
HbA1c, %	-0.1	-0.4†	-0.4†
SBP, mm Hg	-2.1	-2.9	-4.2

\* $P < .0001$  vs placebo; † $P < .05$  vs placebo.

Clinical Trial Registration Number: NCT00553787

Supported by: Vivus, Inc.

## 696

### Effects of extended-release phentermine/topiramate on weight loss and blood pressure in obese subjects with type 2 diabetes mellitus

S.K. Rössner<sup>1</sup>, A.M. Sharma<sup>2</sup>, B. Troupin<sup>3</sup>, R.S. Padwal<sup>4</sup>;

<sup>1</sup>Karolinska University Hospital Huddinge, Bromma, Sweden, <sup>2</sup>Obesity Research & Management, University of Alberta, Edmonton, Canada, <sup>3</sup>Vivus, Inc., Mountain View, USA, <sup>4</sup>Department of Medicine, University of Alberta, Edmonton, Canada.

**Background and aims:** Weight loss (WL) and HbA1c control are essential in the management of type 2 diabetes mellitus (T2DM). The Edmonton Obesity Staging System (EOSS), validated against NHANES data, classifies morbidity based on degree of common weight-related comorbidities. Phentermine plus extended-release topiramate (PHEN/TPM ER) resulted in significant WL vs placebo (PBO) in a Phase 3, double-blind, 56-week study (CONQUER) of overweight/obese subjects with  $\geq 2$  weight-related comorbidities. This post-hoc analysis of subjects with T2DM at baseline evaluated WL based on baseline EOSS category, changes in HbA1c, and net change in percentage of subjects receiving antidiabetic medications (percent subjects increasing-decreasing) across the overall population with T2DM.

**Materials and methods:** CONQUER randomised 2487 subjects to PBO, PHEN 7.5 mg/TPM ER 46 mg (7.5/46), or PHEN 15 mg/TPM ER 92 mg (15/92), 388 (15.6%) of whom had T2DM at baseline ( $n=157$ ,  $n=67$ , and  $n=164$  for PBO, 7.5/46, and 15/92, respectively). In subjects with T2DM, baseline EOSS was scored as follows: 2=established weight-related chronic disease ( $n=347$ ) and 3=established end-organ damage ( $n=41$ ). Diet/exercise or metformin monotherapy were permitted at enrollment. Changes in concomitant drug treatment by physicians blinded to treatment-group assignment were permitted.

**Results:** At week 56 in subjects with T2DM at baseline, greater WL was observed among those receiving PHEN/TPM ER compared with those receiving PBO in both baseline EOSS categories (Table). Significant decreases in HbA1c were also observed with PHEN/TPM ER vs PBO ( $P < .05$ ; Table). In addition, 12.1%, 1.5%, and 0.6% of subjects receiving PBO, 7.5/46, and 15/92, respectively, experienced a net change in antidiabetic medications (percent subjects increasing-decreasing). Discontinuation due to adverse events in subjects with T2DM was 8%, 9%, and 19% for placebo, 7.5/46, and 15/92, respectively, with the most common adverse events being upper respiratory tract infection, constipation and paraesthesia.

**Conclusion:** PHEN/TPM ER treatment significantly reduced weight when compared with PBO, even in comorbid subjects based on EOSS category. This WL was associated with significant improvements in HbA1c in subjects with T2DM.

Table. PHEN/TPM ER effects on weight and HbA1c (T2DM population; ITT-LOCF)

			PBO	7.5/46	15/92
Weight loss from baseline to week 56 by baseline EOSS score	Baseline EOSS score 2 (n=347)	Baseline weight, kg	99.4	97.2	102.4
		Week 56 weight, kg	97.4	90.4	93.6
		LS mean weight loss, %	1.9	6.9*	8.5*
	Baseline EOSS Score 3 (n=41)	Baseline weight, kg	97.9	97.7	109.3
		Week 56 weight, kg	95.6	91.4	97.3
		LS mean weight loss, %	2.3	6.6	10.8†
PHEN/TPM ER effects on HbA1c	Overall population (n=357)	Baseline HbA1c, %	6.8	6.8	6.8
		Week 56 HbA1c, %	6.7	6.4	6.4
		LS mean change in HbA1c, %	-0.1	-0.4†	-0.4†

\* $P < .0001$  vs PBO; † $P < .05$  vs PBO.

Clinical Trial Registration Number: NCT00553787

Supported by: Vivus, Inc.

## 697

### Effects of phentermine and extended-release topiramate alone and in combination on cardiovascular risk factors

J. Jordan<sup>1</sup>, A.V. Astrup<sup>2</sup>, W.W. Day<sup>3</sup>;

<sup>1</sup>Institute of Clinical Pharmacology, Hannover, Germany, <sup>2</sup>Department of Human Nutrition, LIFE, University of Copenhagen, Frederiksberg, Denmark, <sup>3</sup>Vivus, Inc., Mountain View, USA.

**Background and aims:** Obesity is associated with an increased risk of cardiovascular disease. In EQUATE, a 28-week, double-blind, placebo-controlled Phase 3 trial, the cardiovascular efficacy and safety of phentermine (PHEN), extended-release topiramate (TPM ER), and the combination of PHEN/TPM ER were assessed.

**Materials and methods:** In this study, 756 subjects were randomized to placebo ( $n=109$ ), PHEN 7.5 mg monotherapy ( $n=109$ ), PHEN 15 mg monotherapy ( $n=108$ ), TPM ER 46 mg monotherapy ( $n=108$ ), TPM ER 92 mg monotherapy ( $n=107$ ), PHEN 7.5 mg/TPM ER 46 mg ( $n=107$ ), or PHEN 15 mg/TPM ER 92 mg ( $n=108$ ). In addition to the primary efficacy endpoint of percent weight loss at week 28, cardiovascular measures included systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the ITT-LOCF sample and heart rate (HR) in the safety set. Adverse events were also assessed during the trial. Across the entire Phase 3 program, major cardiovascular adverse events were assessed retrospectively.

**Results:** After 28 weeks of treatment, least-squares (LS) mean percent weight loss was greater in all arms vs placebo (Table). In addition, SBP and DBP were improved in all arms vs placebo (Table). In the safety set ( $n=753$ ), HR was decreased in the placebo, TPM ER monotherapy, and combination arms from baseline to week 28 but was increased in the both the PHEN monotherapy arms (Table). In total, 72 (9.6%) subjects discontinued due to any drug-related treatment-emergent adverse event: 6 in placebo, 9 in PHEN 7.5 mg, 8 in PHEN 15 mg, 7 in TPM ER 46, 14 in TPM 92 mg, 10 in PHEN 7.5 mg/TPM ER 46 mg, and 18 in the PHEN 15 mg/TPM ER 92 mg groups. The most common adverse events were upper respiratory tract infections, paraesthesia, and dry mouth. Cardiovascular adverse events were infrequent, with the most common being palpitations.

**Conclusion:** PHEN/TPM ER led to significant weight loss and reduced BP compared with placebo. TPM ER monotherapy and PHEN/TPM ER were both associated with reduced HR whereas subjects receiving PHEN monotherapy experienced a slight increase in HR.



Table. Changes in weight, SBP, DBP, and HR from baseline to week 28

	LS mean change (ITT-LOCF population)		Mean change (safety set)	
	Percent weight loss, %	SBP, mm Hg	DBP, mm Hg	HR, bpm
Placebo	1.7	-1.8	-0.7	-1.9
PHEN 7.5 mg	5.5	-3.3	-1.5	0.9
PHEN 15 mg	6.1	-3.5	-0.9	1.1
TPM ER 46 mg	5.1	-6.8	-2.6	-3.8
TPM ER 92 mg	6.4	-6.4	-3.9	-4.5
PHEN 7.5 mg/TPM ER 46 mg	8.5*	-7.0†	-2.2	-1.6
PHEN 15 mg/TPM ER 92 mg	9.2*	-5.2†	-2.0	-1.6

\* $P < .0001$  vs placebo; † $P < .05$  vs placebo.

Clinical Trial Registration Number: NCT00563368

Supported by: Vivus, Inc.

## 698

### Microarray analysis of diabetic gene expression profile in mouse heart overexpressing adipose differentiation-related protein

J. Suzuki<sup>1</sup>, M. Ueno<sup>1</sup>, M. Ichikawa<sup>1</sup>, M. Imagawa<sup>1</sup>, S. Sato<sup>1</sup>, Y. Zenimaru<sup>1</sup>, T. Koizumi<sup>2</sup>, S. Ikuyama<sup>3</sup>, S. Takahashi<sup>1</sup><sup>1</sup>Endocrinology and Metabolism, University of Fukui, Faculty of MedicalScience, Eihei, <sup>2</sup>Laboratory Animal Resources, University of Fukui, Eihei, <sup>3</sup>The medical institute of bioregulation, University of Kyushu, Oita, Japan.

**Background and aims:** Diabetic cardiomyopathy is characterized by intracellular lipid accumulation (steatosis) that generates lipotoxicity and eventually leads to cardiac dysfunction. The adipose differentiation-related protein (ADRP) is a lipid droplet-associated molecule expressed in many tissues including heart. Since we found that the expression of ADRP was dysregulated in diabetic heart, we have created heart-specific ADRP-overexpressing (Tg) mice to study function of cardiac ADRP in diabetic condition. We have reported in 2011 session that despite massive steatosis, Tg mice maintained normal cardiac function with decreased expression of fatty acid-responsive genes. In the current study we further analyzed cardiac gene expression profile using microarray to address the pathophysiology of lipotoxic cardiomyopathy.

**Materials and methods:** Tg mice and wildtype (Wt) littermates were treated with streptozotocin to induce diabetes. The hearts were collected 3-weeks afterwards and gene expression profile was analyzed using Affymetrix microarrays. Comparison analyses were performed among 4 groups; control Wt, diabetic Wt, control Tg and diabetic Tg mice, and the selected genes were analyzed by qRT-PCR. Pathway analysis for the altered genes was performed using KEGG database, and gene ontology (GO) classification was carried out through GO Slim database.

**Results:** The induction of diabetes altered (>2-fold) expression of 810 genes in Wt hearts and 1141 genes in Tg hearts. In Wt hearts 17 pathways were identified to be affected significantly by the induction of diabetes through KEGG pathway analysis. The 17 pathways included PPAR signaling, Ca signaling, and dilated cardiomyopathy. In contrast, in Tg hearts only 2 pathways out of the 17 pathways were identified to be affected. Importantly, 5 genes involved in PPAR signaling were differentially expressed with diabetes in Tg hearts compared to Wt hearts. GO classification identified 12 unique functional groups induced by diabetes in Tg hearts. Among them, gene group of cell motility and homeostatic process were differentially regulated in Tg hearts compared with Wt hearts.

**Conclusion:** Packaging excess fatty acids into lipid droplets, cardiac ADRP might play a role in counteracting diabetes-induced gene expression in heart. Thus, cardiac ADRP could be a target to regulate cardiac lipotoxicity in diabetes.

Supported by: JSPS

## PS 052 Diabetes and cancer

### 699

#### Pre-existing type 2 diabetes and mortality of cancer patients in Latvia

I. Strele<sup>1</sup>, S. Pildava<sup>2</sup>, G. Brigis<sup>1</sup><sup>1</sup>Riga Stradins University, <sup>2</sup>National Health Service, Riga, Latvia.

**Background and aims:** Studies show that cancer patients with pre-existing diabetes experience higher all-cause mortality compared to non-diabetic cancer patients. Explanations of that association vary from timely detection of cancer to different cancer treatment practices and side-effects. We compared all-cause mortality between cancer patients with and without prior type 2 diabetes, considering also the cancer stage at diagnosis.

**Materials and methods:** The data source was two nationwide Cancer and Diabetes Registers. All patients from 2005 to 2009 with the first incident tumor were selected and their diabetes status ascertained. Patients with type 1 and other specific types of diabetes were excluded, as well as cases in which diabetes and cancer occurred in the same year. Thus, the study included 25,180 male and 26,906 female patients with cancer, of those 1,493 and 2,383, respectively, had type 2 diabetes prior the diagnosis of cancer. Cox regression was used to assess the all-cause mortality difference, shown as a hazard ratio (with 95%CI) with non-diabetic cancer patients as the reference group. As diabetes patients were older, both among men (mean age 69.6 vs. 64.9 years) and women (mean age 71.0 vs. 64.0 years), the estimates presented were adjusted for age at cancer diagnosis.

**Results:** Among men, cancer patients with diabetes had an even lower mortality within the first five follow-up years than cancer patients without diabetes: HR 0.84 (0.78-0.90),  $p < 0.001$ . Analysis according to specific cancer site resulted in a few statistically significant findings. Among men with lung and tracheal cancer, prior diabetes was associated with lower mortality: HR 0.83 (0.69-0.98),  $p = 0.026$ . In the case of prostate cancer, patients with pre-existing diabetes had increased mortality, but only after the first five years of follow-up: HR 2.42 (1.19-4.89),  $p = 0.014$ . Further adjustment for cancer stage did not substantially change these estimates. Among women, cancer patients with diabetes, overall, experienced a mortality similar to that of cancer patients without diabetes: HR 1.06 (0.99-1.13),  $p = 0.055$ ; however, the difference increased after adjusting for cancer stage: HR 1.10 (1.03-1.16),  $p = 0.003$ . Specifically, this pattern was observed in women with colon or breast cancer: HR when adjusted only for age was 1.16 (0.95-1.40),  $p = 0.141$  in the case of colon cancer and 1.02 (0.86-1.22),  $p = 0.784$  in the case of breast cancer; although, after adjustment for stage, it became 1.25 (1.02-1.52),  $p = 0.025$  and 1.19 (1.01-1.42),  $p = 0.044$ , respectively. But in ovary cancer, association of diabetes and mortality changed over time. If mortality risk was slightly increased and not statistically significant within the first 1.5 years, then in women who survived the first 1.5 years since diagnosis, pre-existing diabetes was associated with better subsequent survival: HR 0.46 (0.25-0.84),  $p = 0.011$ .

**Conclusion:** In Latvia, cancer patients with prior type 2 diabetes do not experience increased all-cause mortality, at least within the first five years since cancer diagnosis. Overall, cancers in the diabetic population were not diagnosed at more advanced stages, but in women with diabetes, even an earlier cancer stage was responsible for survival similar to that of non-diabetic women. As diabetes patients regularly contact the health care system, then, probably, low screening uptake and late cancer detection in the general population reduce this difference.

### 700

#### Cancer mortality among Danish diabetic patients compared to patients without diabetes

K. Ranc<sup>1</sup>, M.E. Jorgensen<sup>1</sup>, D.R. Witte<sup>1</sup>, B. Carstensen<sup>1,2</sup><sup>1</sup>Steno Diabetes Center, <sup>2</sup>Department of Biostatistics, University of Copenhagen, Denmark.

**Background and aims:** It is known that cancer patients with pre-existent diabetes experience higher mortality compared to cancer patients without diabetes. However, the role of different diabetes treatment types has not been studied in detail. We compared mortality rates among cancer patients with and without diabetes, accounting for treatment.

**Materials and methods:** The study is based on all cancer patients diagnosed in Denmark in the period 1995-2009. We linked the Danish Cancer Register (DCR) and the Danish National Diabetes Register (NDR). Cancer patients were classified into 4 groups according to diabetes status at the time of diag-

nosis of cancer: no diabetes, diabetes without medication, diabetes with only OAD treatment or diabetes with Insulin treatment. Cox proportional hazard models were used to estimate sex and age adjusted cancer mortality hazard-ratios relative to non-diabetic cancer patients.

**Results:** Among 426,129 new cancer cases, we found 42,484 that had diabetes prior to the cancer diagnosis (14,315 without medication history, 17,699 on OADs and 8,582 on Insulin). Over all, diabetes patients had a higher mortality rate compared to non-diabetic cancer patients. Cancer patients with insulin treated diabetes had a 48% (95%CI: 1.45–1.52) higher mortality, those with OAD treated diabetes had a 23% (95%CI: 1.21–1.26) higher mortality, and those with diabetes without medication had a 13% (95%CI: 1.11–1.15) higher mortality than non-diabetic cancer patients.

**Conclusion:** Based on a nation-wide register linkage study, we confirmed that cancer patients with pre-existing diabetes experience higher mortality than cancer patients without diabetes. The higher mortality seen among cancer patients with OAD and insulin treated diabetes respectively is in accordance with the notion that more intensive diabetes treatment reflects a larger degree of co-morbidity at the time of cancer diagnosis, and hence poorer survival.

## 701

### Postprandial blood glucose predicts cancer mortality in type 2 diabetes mellitus: lessons from the 14-year follow-up of the San Luigi Gonzaga diabetes study

F.L. Cavalot, A. Pagliarino, M. Valle, L. Di Martino, K. Bonomo, P. Poy, C. Vaccheris, M. Trovati;

Dept of Clinical and Biological Sciences, University of Turin, Orbassano, Italy.

**Background and aims:** Type 2 diabetes mellitus (T2DM) has been associated with a variety of neoplasms. Among the factors that have been involved in this relationship, obesity, insulin-resistance, hyperinsulinemia and type of therapy should be mentioned. If blood glucose (BG) control plays any role in predicting cancer mortality in T2DM has not been clarified so far; furthermore, if postprandial blood glucose (PPBG), that we and others have shown to predict cardiovascular events and total mortality, also predicts cancer mortality is completely unknown. Aim of the present study was to evaluate the predictive power of HbA1c, fasting BG (FBG) and PPBG on cancer mortality in a long-term follow-up of patients affected by T2DM.

**Materials and methods:** We studied, in a 14-year follow-up, 622 T2DM patients (M/F 340/282, age:  $62.1 \pm 9.4$  years, known diabetes duration:  $9.6 \pm 7.9$  years) belonging to the San Luigi Gonzaga Diabetes Study cohort in Turin, Piedmont, North West of Italy. In these patients, at the enrollment in 1995 data were collected concerning glycated haemoglobin HbA1c, FBG, BG 2 hours after lunch (PPBG) and the main cardiovascular risk factors. Subjects were followed for many outcomes, among them the cause of death. We performed Cox analysis with the backward method in order to evaluate the predictive power for cancer mortality of HbA1c, FBG, and PPBG measured at baseline and adjusted for age, gender, known diabetes duration, smoking habit, body mass index, systolic and diastolic blood pressure, total and HDL cholesterol, triglycerides, albumin excretion rate and serum creatinine. We built four models, in which all the non-glycaemic variables were included as correctors, whereas the glycaemic variables were included as follows: Model A, HbA1c; Model B, FBG; Model C, PPBG; Model D, HbA1c, FBG and PPBG. The glycaemic variables were inserted in the Models as continuous values.

**Results:** At the follow-up, 192/622 patients died, 49 as a consequence of cancer. In all Models, age and smoking habit resulted to be the significant non-glycaemic predictors. In Model A, the HR per 1% HbA1c was 1.241, CI 1.015–1.517,  $p=0.036$ ; in Model B, FBG was not a predictor, with HR per 1 mmol/L 1.066, CI 0.965–1.177,  $p=ns$ ; in Model C, the HR per 1 mmol/L PPBG was 1.108, CI 1.021–1.202,  $p=0.014$ ; in Model D, the only significant glycaemic predictor was PPBG, its HR per 1 mmol/L being 1.108, CI 1.021–1.202,  $p=0.014$  (as in Model C).

**Conclusion:** Blood glucose control is a predictor of cancer mortality in T2DM. Among the glycaemic variables considered, FBG does not predict cancer mortality, HbA1c is a predictor only in Models that do not include also PPBG, while PPBG is a predictor by itself and remains the only glycaemic predictor of cancer mortality when included together with FBG and HbA1c in the statistical Models. On the basis of these data, we can conclude that PPBG is the most relevant component of blood glucose control to play a predictive role for cancer mortality in T2DM.

Supported by: Piedmont Region Grants to FC and MT

## 702

### Interrelation between prevalence rates of diabetes and of malignant neoplasms evaluated by crossing different databases: a retrospective study

F. Bardelli<sup>1</sup>, F. Tesi<sup>2</sup>, R. Anichini<sup>2</sup>, G. Seghieri<sup>2</sup>;

<sup>1</sup>Pharmaceutical Service Dpt., <sup>2</sup>Dpt. of Internal Medicine, Pistoia, Italy.

**Background and aims:** The relationship between cancer and diabetes has been for a long time well-known, and the upward trend of prevalence/incidence rates of both diabetes and malignant neoplasms (MN) is likewise known. With the purpose of obtaining indirect information about relationship between rates of MN and of diabetes in our population we have utilized the pharmaceutical registry databases (PRD) of local health area which covers a population of about 280,000 inhabitants.

**Materials and methods:** PRD contains records of all patients who received prescriptions for antidiabetic drugs (oral and insulin), which are totally reimbursed by the Regional Health System as well as records of patients exempted from payment for medical services because affected by neoplastic diseases. Consequently PRD has been screened across a 6-years-period from 2006 to 2011 to obtain a reasonable proxy esteem of time-related interrelation between prevalence of diabetes and of MN.

**Results:** Population rose from 279,061 inhabitants (134,340M/144,721F) in 2006 to 293,061 (140,626M/152,435F) in 2011, and during this period we observed a parallel increase in the rate of patients who were given prescriptions for antidiabetic drugs, expressed as % of total population, from 4.06% to 4.47% (from 4.23% to 4.48% in males and from 3.90% to 4.18% in females). Likewise we also observed a progressive increment of those exempted from payment for medical services because affected by neoplastic diseases from 2.23% in 2006 to 3.38% in 2011. Within the group of treated diabetic patients we noticed a progressive increase in prevalence of neoplastic patients from 508 (4.93%), in January 2006 to 868 (6.63%) in December 2011, and specularly there was an upward trend for prevalence of diabetic patients in the group of neoplastic patients (7.9% in 2006, 8.5% in 2007, 8.8% in 2008, 9.2% in 2009, 9.4% in 2010 and 9.6% in 2011;  $p=0.0001$  for trend test). However relative risk (95%CI) of co presence of diabetes and MN remained similar in 2006 and in 2011 [2.23 (1.13–2.89) in 2006 and 2.06 (1.14–2.92)] in 2011. Throughout the entire period the ratio M/F was  $>1$ , among diabetics, while remained persistently  $<1$  in neoplastic patients whether diabetic or not.

**Conclusion:** According to this preliminary study crossing data from different PRD databases allows us to suggest that: a) both prevalence of neoplastic disease in diabetic patients and prevalence of diabetes in neoplastic patients are progressively growing with time, b) in spite of increasing rates of treated diabetes and of patients with exemption for MN relative risks of co presence of both pathologies remain constant with time, with diabetic patients presenting a twice higher relative risk for MN, and c) female gender predominates among neoplastic patients whether they are suffering from diabetes or not.

Supported by: Fondazione Cassa di Risparmio di Pistoia e Pescia

## 703

### Insulin resistance as a link between type 2 diabetes mellitus and colon cancer

P.J. Piattkiewicz<sup>1</sup>, A. Czech<sup>1</sup>, T. Milek<sup>2</sup>, M. Bernat-Karpinska<sup>1</sup>, A. Górski<sup>3</sup>;

<sup>1</sup>Chair and Department of Internal Diseases and Diabetology, <sup>2</sup>Chair and Department of General and Vascular Surgery, <sup>3</sup>Department of Clinical Immunology, Warsaw Medical University, Poland.

**Background and aims:** In subjects with type 2 diabetes there is a more significant occurrence of colon cancer. The aim of this study was to evaluate the possible differences in glucose metabolism parameters and insulin resistance indicators in Type 2 diabetic patients with negative family history of cancer (T2DP), Type 2 diabetic patients with newly diagnosed untreated colon cancer (T2DCCP), and subjects without diabetes with newly diagnosed, untreated colon cancer (CCS). The specific objective of this study was to verify the hypothesis that the positive relationship between type 2 diabetes and colon cancer may be associated with the applied method of type 2 diabetes treatment.

**Materials and methods:** 18 T2DP treated with diet and oral antidiabetic agents (metformin or sulfonylurea), 19 T2DCCP cT1-4N0M0 (c-clinical diagnosis based on computer tomography, colonoscopy and histopathology) treated with diet and oral antidiabetic agents (metformin or sulfonylurea), and 20 normoglycemic CCS cT1-4N0M0 were enrolled into the study. The operation was conducted in a period of up to 14 days after the clinical diagno-

sis had been made. Histopathological results verified the clinical diagnosis. In all analyzed cases, the presence of adenocarcinoma was registered. Due to the finding of metastases in mesenteric lymph nodes, 4 patients were excluded from the CCS group and 3 patients from the T2DCCP group. The control group included 18 metabolically healthy subjects (HS) with negative family history of cancer, matched with age, BMI and waist circumference.

**Results:** Duration of diabetes was similar in T2DP and T2DCCP respectively ( $6,3 \pm 2,9$  vs.  $6,5 \pm 3,0$ ). However T2DCCP differed significantly from T2DP in terms of therapy of type 2 diabetes. In T2DP the predominant anti-diabetic drug was metformin, which was used in 83.3% of patients. 75% of T2DCCP were treated with a sulfonylurea. The anthropometric and biochemical parameters of the study groups are presented in the table. (Table).

**Conclusion:** This study indicates a positive and statistically significant correlation between the high levels of endogenous insulin in plasma and an increased risk of colon cancer. Our study also shows that metformin intake reduces the risk of colon cancer. On the contrary, the mode of therapy for type 2 diabetes, which is directly linked to augmenting endogenous insulin levels, increases the risk of colon cancer. Thus, the kind of treatment applied for type 2 diabetic patients may modify the risk of colon cancer. The frequent screening tests towards colon cancer in subjects with type 2 diabetes, especially in those with coexisting insulin resistance seems to be of a great purpose.

Table (p-values are related to HS group).

PARAMETER (x ± SD)	HS	T2DP	CCS	T2DCCP
NUMBER	18	18	16	16
SEX (M/F)	8 / 10	9 / 9	7 / 9	8 / 8
AGE (years)	52,4 ± 7,0	53,6 ± 7,0	55,2 ± 8,0	55,0 ± 8,0
BMI (kg/m <sup>2</sup> )	26,3 ± 3,0	26,7 ± 5,1	25,7 ± 3,0	26,1 ± 3,0
WHR	0,87 ± 0,09	0,88 ± 0,08	0,87 ± 0,09	0,88 ± 0,09
FASTING PLASMA GLUCOSE (mmol/l)	5,37 ± 0,68	7,20 ± 0,67 <i>p</i> <0,01	5,46 ± 0,74 ns	7,45 ± 0,95 <i>p</i> <0,01
HbA1c (%)	5,4 ± 0,7	7,13 ± 0,61 <i>p</i> <0,01	5,45 ± 0,61 ns	7,17 ± 0,93 <i>p</i> <0,01
FASTING INSULIN (mU/l)	7,89 ± 3,65	14,04 ± 1,59 <i>p</i> <0,01	8,12 ± 4,63 ns	20,91 ± 8,09 <i>p</i> <0,01
HOMA-IR	1,88 ± 1,03	4,47 ± 1,98 <i>p</i> <0,01	1,97 ± 1,1 ns	6,92 ± 2,8 <i>p</i> <0,01

Supported by: Warsaw Medical University

## 704

### Comparative assessment of event rates of bladder cancer and nine other cancers in patients with type 2 diabetes mellitus treated with pioglitazone or insulin

C. Vallarino<sup>1</sup>, J. Yang<sup>2</sup>, A. Perez<sup>1</sup>, G. Fusco<sup>1</sup>, H. Liang<sup>1</sup>, M. Bron<sup>2</sup>, V. Harikrishnan<sup>2</sup>, G. Joseph<sup>1</sup>, S. Yu<sup>2</sup>

<sup>1</sup>Takeda Global Research & Development Center, Inc., Deerfield, USA,

<sup>2</sup>Takeda Pharmaceuticals International, Inc., Deerfield, USA.

**Background and aims:** To address concerns about a possible increased risk of bladder cancer in type 2 diabetes mellitus (T2DM) patients receiving pioglitazone, a retrospective cohort study was designed to assess the event rates of bladder cancer and a composite of nine other selected cancers (prostate, female breast, lung, pancreatic, endometrial, non-Hodgkin's lymphoma, colorectal, kidney and malignant melanoma) among new users of pioglitazone (PIO) or insulin (INS).

**Materials and methods:** Data from May 1, 2000 to June 30, 2010 were extracted from the i3 InVision Data Mart, a US medical and prescription records database representing approximately 47 million covered lives during the period of analysis. Key outcomes were incident cases of bladder cancer and incident cases of nine other cancers. As pre-planned additional analyses, a composite endpoint of all-cause mortality and bladder cancer and a composite endpoint of all-cause mortality and nine other cancers were also created using death records obtained from the US Social Security Administration in March 2012. Kaplan-Meier curves were generated and hazard ratios (HR) estimated from Cox proportional hazards models adjusted with inverse probability weights derived from propensity scores.

**Results:** A total of 56,536 patients (PIO group: 38,588; INS group: 17,948) aged ≥45 years qualified for the study (mean age: 58.1 and 59.7 years, male gender: 59.6% and 53.0%, mean follow-up: 2.2 and 1.9 years, for PIO and INS; respectively). For bladder cancer, the incidence rate showed no significant difference between the two groups; the HR for PIO vs INS was 0.92 (95% con-

fidence interval (CI) [0.63, 1.33], *p*=0.64). The results were similar for bladder cancer with additional confirmation based on cancer-related procedures, which served as a sensitivity check. There was no evidence of the incidence rate of bladder cancer increasing with time of exposure to PIO. Cox regression results for the composite of nine other cancers yielded an HR for PIO vs INS of 0.78 (95% CI [0.71, 0.85], *p*<0.0001). The raw incidence rates of nine other cancers for PIO and INS were 1798 and 2456 per 100,000 person years, respectively, which were 16 times higher than the rate of bladder cancer alone (PIO: 113; INS: 152). The risk of all-cause mortality plus bladder cancer was lower in the PIO group compared with the INS group; the HR for PIO vs INS was 0.40 (95% CI [0.36, 0.44], *p*<0.0001). Similarly, the risk of all-cause mortality plus nine other selected cancers was also lower in the PIO group; the HR for PIO versus INS was 0.58 (95% CI [0.54, 0.62], *p*<0.0001).

**Conclusion:** The results indicate that, compared to INS, PIO was not associated with an increased risk of bladder cancer. Additionally, PIO was associated with a significantly lower risk of developing other selected cancers. When all-cause mortality was included in the analysis, the risks of all-cause mortality plus bladder cancer and all-cause mortality plus nine other selected cancers were both considerably lower in the PIO group than in the INS group. Supported by: Takeda Pharmaceuticals International, Inc.

## 705

### Metformin prevents high-fat diet-induced liver tumorigenesis in C57BL/6J mice

K. Tajima, A. Nakamura, J. Shirakawa, K. Orime, Y. Togashi, Y. Nagashima, Y. Terauchi;  
Yokohama City University, Japan.

**Background and aims:** We previously demonstrated that long-term high-fat (HF) diet loading induced NASH and liver tumorigenesis in C57BL/6J mice, and that liver fat accumulation was involved in the development of tumorigenesis. Metformin, one of the most widely used oral hypoglycemic agents, has recently increased attention because of its potential anti-tumor effects. Here, we investigated the effects of metformin on HF diet-induced liver tumorigenesis using mouse model of NASH and liver tumorigenesis.

**Materials and methods:** We challenged 8-week-old C57BL/6J male mice with the standard chow diet (SC group), a HF diet (HF group) or a HF diet with treatment of metformin in drinking water (250 mg/kg/day) (HF+Met group) for 60 weeks. We also treated HF diet-induced NASH model mice with metformin. Eight-week-old C57BL/6J mice were administered the HF diet for 30 weeks, followed by HF diet with metformin for another 30 weeks (HF-HF+Met group) and they were compared with mice on the HF diet throughout the 60 weeks (HF-HF group). The histological and phenotypical analyses were performed.

**Results:** After 60 weeks, the HF group showed significantly increased body weight, liver weight and plasma alanine aminotransferase (ALT) levels as compared with the SC group. Although the body weight of HF+Met group was comparable to the HF group, metformin treatment significantly decreased liver weight and plasma ALT levels. The triglyceride (TG) content in the liver was comparable between the HF and HF+Met group. Scoring of the pathological findings showed a significant increase in the scores for liver steatosis, inflammation and fibrosis in the HF group as compared with the SC group. Of note, metformin treatment significantly improved the scores for inflammation and fibrosis, but not steatosis compared with the HF group. The incidence of the nodular lesions on the liver surface was significantly decreased in the HF+Met group as compared with the HF group (25% vs. 70%). We next investigated whether metformin prevented NASH model mice that had been administered HF diet for 30 weeks from the development of liver tumors. The body weight, liver weight and TG content in the liver were comparable between the HF-HF and HF-HF+Met group. Moreover, the incidence of nodular lesions on the liver surface was not different between HF-HF group and HF-HF+Met group (75% vs. 83%). We next assessed the short-term effect of metformin on HF diet-induced liver fat accumulation. Hepatic histopathology and TG levels showed that hepatic triglyceride content was larger in the HF group after 8 weeks than in the SC group, and was decreased by metformin treatment. With regard to adipose tissue function, the adipocyte size of the epididymal fat in the HF group was larger than in the SC group, and was decreased by metformin treatment. In addition, the mRNA expression levels of F4/80 and CD11c were significantly higher in the HF group than the SC group, whereas they were decreased by metformin treatment.

**Conclusion:** Metformin prevented HF diet-induced liver tumorigenesis in C57BL/6J Mice. On the contrary, additional treatment of metformin failed to



reduce the incidence of liver tumorigenesis. These results suggested that the mechanism underlying anti-tumour effect of metformin was at least partly associated with suppression of liver fat accumulation at early stage. Suppression of liver fat accumulation by metformin may be associated with the prevention from adipose tissue dysfunction by HF-feeding, but further study is needed to address this association.

## 706

### The effect of rhIGF-1 and insulin analogue AspB10 on mammary tumour growth and progression in a mouse model of type 2 diabetes

E.J. Gallagher<sup>1</sup>, N. Alikhani<sup>1</sup>, A. Tobin-Hess<sup>1</sup>, D. Cannata<sup>1</sup>, N. Tennagels<sup>2</sup>, U. Werner<sup>2</sup>, D. LeRoith<sup>1</sup>;

<sup>1</sup>Samuel Bronfman Dept of Medicine, Mount Sinai School of Medicine, New York, USA, <sup>2</sup>R&D Diabetes Division, Sanofi, Frankfurt, Germany.

**Background and aims:** Epidemiological studies have clearly demonstrated the association between obesity, type 2 diabetes (T2D) and an increased risk of developing cancer and cancer-related mortality. This may be attributable to hyperinsulinemia, hyperglycemia, hyperlipidemia or factors such as leptin, IGF-1 and inflammatory cytokines. One of the malignancies adversely affected by obesity and T2D is breast cancer. The MKR mouse is a model of T2D. The females are hyperinsulinemic, lean, display a mild dysglycemia and have normal circulating IGF-1 levels. The female MKR mice have been utilized in our previous studies to demonstrate the role of endogenous hyperinsulinemia in mammary tumor growth, without the confounding variable of obesity. In the present study, we are investigating the effect of IGF-1 and human insulin analog AspB10, in addition to endogenous hyperinsulinemia, on mammary tumor growth and progression.

**Materials and methods:** Non-metastatic Met-1 and metastatic Mvt-1 mammary cancer cells derived from MMTV-PyVmT/FVB-N transgenic mice and c-Myc/vegf tumor explants respectively, were inoculated into the 4th mammary fat pad of MKR females. Mice were divided into 2 groups matched for tumor size and were treated with rhIGF-1 (2mg/kg/day i.p.) or vehicle for 14 days in the initial study, and with AspB10 (12.5IU/kg twice daily s.c.) or vehicle for 14 days in the second study.

**Results:** Treating the MKR mice with rhIGF-1 and AspB10 insulin analog respectively, resulted in significantly increased tumor growth, when compared to vehicle treated MKR mice, as measured by tumor volume over a 28 day period. (Mvt-1 tumors: 170 mm<sup>3</sup> in rhIGF-1 treated vs 68 mm<sup>3</sup> in vehicle treated; Met-1 tumors: 55 mm<sup>3</sup> in rhIGF-1 treated vs 31 mm<sup>3</sup> in vehicle treated; Mvt-1 tumors: 144 mm<sup>3</sup> in AspB10 treated vs 73 mm<sup>3</sup> in vehicle treated; Met-1 tumors: 72 mm<sup>3</sup> in AspB10 treated vs 35 mm<sup>3</sup> in vehicle treated,  $p < 0.05$ ).

**Conclusion:** These results indicate an independent effect of rhIGF-1 and AspB10 in mammary tumor growth in the setting of a hyperinsulinemic non-obese environment. We are currently investigating the receptor and signaling pathway through which IGF-1 and AspB10 promote mammary tumor growth in the hyperinsulinemic milieu.

*Supported by: Sanofi*

## 707

### Effects of the inhibition of insulin degrading enzyme (IDE) activity on the liver cancer cells transcriptome

N.N. Rudovich<sup>1,2</sup>, O. Pivovarova<sup>1,2</sup>, I. Ilkavets<sup>3</sup>, C. Sticht<sup>4</sup>, S. Zhuk<sup>5</sup>, A. Malashicheva<sup>5</sup>, C. Bumke-Vogt<sup>1</sup>, S. Lukowski<sup>3</sup>, A. Schlag<sup>3</sup>, A. Kostareva<sup>5</sup>, N. Gretz<sup>4</sup>, S. Dooley<sup>3</sup>, A.F.H. Pfeiffer<sup>1,2</sup>;

<sup>1</sup>Clinical Nutrition, DIFE Potsdam, Nuthetal, Germany, <sup>2</sup>Campus B. Franklin, Charite University of Berlin, Berlin, Germany, <sup>3</sup>Molecular Hepatology - Alcohol Associated Diseases, Medical Clinic, Faculty of Medicine Mannheim at Heidelberg University, Germany, <sup>4</sup>Center for medical research (ZMF), Faculty of Medicine Mannheim at Heidelberg University, Mannheim, Germany, <sup>5</sup>Institute of Molecular Biology and Genetics, Almazov Federal Heart, Blood and Endocrinology Center, Saint-Petersburg, Russian Federation.

**Background and aims:** Type 2 diabetes mellitus (T2DM) characterized by hyperinsulinemia is an independent risk factor for development and progression of hepatocellular carcinoma. Insulin degrading enzyme (IDE) is a major enzyme responsible for insulin degradation in the liver. We and others demonstrated that metabolic modulation of IDE activity is superior compared to its alteration at protein level. Inhibition of IDE activity results in increased intracellular insulin concentration, which might be crucial for insulin-depend-

ent regulation of gene expression and cell proliferation. Therefore, modulating IDE activity may represent a key link between T2DM and liver cancer. Here, we characterized variations in the gene expression of HepG2 hepatoma cell line upon modulation of IDE activity.

**Materials and methods:** We analyzed the HepG2 cell transcriptome under four conditions: in untreated cells or after insulin treatment (10 nM insulin for 24 h) with or without inhibition of IDE activity. To modulate IDE activity in HepG2 cells, known IDE inhibitors (bacitracin, N-ethylmaleimide, 1,10-phenanthroline) or IDE silencing (RNAi) were used. Gene expression profiling was performed of HUGene-1.0-st-v1-type from Affymetrix which allows monitoring of expression of about 19 000 transcripts. In addition, we measured mRNA expression of IDE gene with Real Time PCR in liver normal and cancer cells lines with different proliferative capacity (HepG2, HCC-M, HCC-T, Hep3B, HLF, PLC4, FLC4, HLE and Huh7).

**Results:** All used chemical inhibitors demonstrated toxicity for HepG2 cells in concentrations, which are effective to inhibit IDE activity according to literature data. Therefore, only IDE RNAi was used in experiments for transcriptome analyses. The IDE-knockdown regulated a greater part of transcriptome in comparison with insulin effects (0.05% vs 0.71% by  $p < 0.05$  with FDR-correction). In basal state, IDE silencing led to the regulation of genes related to MAPK and p53 signaling pathways as well as endocytosis and ligand-receptor interactions. Insulin treatment in IDE knockdown cells resulted in expression alterations of cell cycle genes that were not detected in control cells. Moreover, proliferation markers MKI67, MCM2 and PCNA were significantly upregulated upon RNAi for IDE. In Huh7 and HCC-T cells IDE gene expression was strong down regulated.

**Conclusion:** Inhibition of IDE activity can lead to insulin mediated upregulation of proliferation pathway genes in liver cells. Thus, IDE becomes a potential target for therapeutic intervention in T2DM and cancer.

*Supported by: German Diabetic Association*

## 708

### Metformin attenuates proteasome 26S activity and exerts tumour-suppressing effects in human hepatoma cells

A. Obara, Y. Fujita, A. Abudukadier, T. Fukushima, M. Hosokawa, N. Inagaki; Department of Diabetes and Clinical Nutrition, Graduate School of Medicine, Kyoto University, Japan.

**Background and aims:** Metformin is widely prescribed as an oral anti-hyperglycemic drug for treatment of type 2 diabetes, and metformin comes to the front by its anti-carcinogenic effect. This effect is induced by suppression of mTOR signaling in breast and prostate cancer cells. Recent studies have shown that mTOR signaling is regulated by the proteasome degradation system. Although the major target tissue of metformin is liver and several clinical studies show that metformin reduces the risk of hepatocellular carcinoma and lowers mortality in diabetic patients, the effect of metformin in suppressing cell proliferation in liver remains unknown. The aim of this study was to investigate the effect of metformin on cell proliferation and the role of the proteasome degradation system in liver cancer cells.

**Materials and methods:** HepG2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5.6 mmol/l glucose supplemented with 10 % (vol./vol.) fetal bovine serum. HepG2 cells were incubated in DMEM treated with metformin, 5-Aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranosyl 5'-monophosphate (AICAR), regular insulin, or proteasome inhibitor (MG132). The media were changed every 24 hours. After 72 hours incubation, cell proliferation assay and proteasome activity assay were performed. Cell proliferation rate was determined by MTT assay, absorbance was measured at 490 nm. Proteasome 26S activity was measured by fluorogenic proteasome substrate (N-Succinyl-Leu-Leu-Val-Tyr-7-Amido-4-Methylcoumarin) assay, and absorbance was measured at 360 nm/460 nm. Protein expressions of AMP-activated protein kinase alpha (AMPK alpha), phospho-AMPK alpha, phospho-p70 S6 kinase, and DEPTOR were analyzed by western blotting.

**Results:** Metformin and AICAR significantly suppressed cell proliferation of HepG2 cells over 72-hour incubation. MG132, a proteasome inhibitor, also suppressed cell proliferation, but insulin did not (metformin,  $0.601 \pm 0.032$ ; MG132,  $0.495 \pm 0.011$ ; AICAR,  $0.596 \pm 0.022$ ; insulin,  $0.782 \pm 0.044$ ; control,  $0.836 \pm 0.030$ ; absorbance at 490 nm;  $p < 0.001$  vs control). Metformin attenuated proteasome 26S activity (metformin,  $57.8 \pm 5.7$  % of control;  $p < 0.001$ ). Activation of p70-S6 kinase, a downstream target of mTOR signaling, was suppressed in the presence of metformin. We then investigated the target molecule associated with the mTOR system that is degraded by the proteasome system. As a candidate, we evaluated the expression of DEPTOR, an

mTOR suppressing substrate known to be degraded by the proteasome system, and found that it was increased in the presence of metformin.

**Conclusion:** Metformin suppressed cell proliferation of HepG2 cells. The proteasome degradation system may have an important role in suppression of cell proliferation of liver cancer by metformin.

## PS 053 Glucagon-related peptides and receptors

### 709

#### Effects of liraglutide on gastric emptying and postprandial glucose metabolism in obese, non-diabetic adults: a randomised placebo-controlled incomplete crossover trial

E. Blaak<sup>1</sup>, J. van Can<sup>1</sup>, B. Sloth<sup>2</sup>, C.B. Jensen<sup>2</sup>, T.D. Le Thi<sup>2</sup>, A. Flint<sup>2</sup>, W.H.M. Saris<sup>1</sup>;

<sup>1</sup>Maastricht University, Netherlands, <sup>2</sup>Novo Nordisk A/S, Søborg, Denmark.

**Background and aims:** This study investigated the effects of once-daily s.c. liraglutide, a human GLP-1 analogue, on gastric emptying (GE) and postprandial (PP) glucose metabolism in obese adults without type 2 diabetes (T2D). Liraglutide 1.8 mg is approved for treatment of T2D while liraglutide 3.0 mg is under development for weight management.

**Materials and methods:** Subjects (N=49, age 18–75 years, BMI 30–40 kg/m<sup>2</sup>) were randomised to once-daily liraglutide 3.0 mg, 1.8 mg, or placebo in a double-blind, incomplete crossover design. GE (paracetamol absorption method) and glycaemic parameters were assessed during a 5-hour meal test after 5 weeks of treatment.

**Results:** Equivalence in GE (AUC<sub>0–300min</sub>; primary endpoint) was observed for liraglutide 3.0 mg vs. 1.8 mg (90% CI contained within [0.80;1.25]) and liraglutide vs. placebo. However, 1-hour GE (AUC<sub>0–60min</sub>) decreased by 23% with liraglutide 3.0 mg (ratio 0.77, 95%CI [0.6;0.9], p=0.007) and 13% with 1.8 mg (ratio 0.87, 95%CI [0.7;1.1], p=0.14) vs. placebo. Liraglutide 3.0 mg and 1.8 mg reduced fasting plasma glucose by 0.5 mmol/L vs. placebo (p<0.0001); no effects on fasting glucagon, insulin or C-peptide were seen. Incremental AUC (iAUC<sub>0–300min</sub>) for glucose decreased with liraglutide 3.0 mg (p=0.02) but not with 1.8 mg (p=0.99) vs. placebo. PP glucagon (iAUC<sub>0–300min</sub>) decreased by about 30% with both liraglutide 3.0 mg (p=0.051) and 1.8 mg (p=0.04). Liraglutide 3.0 mg and 1.8 mg significantly lowered 1-hour PP insulin (iAUC<sub>0–60min</sub>) by 22–26% and C-peptide by 18–21% vs. placebo.

**Conclusion:** Liraglutide 3.0 mg and 1.8 mg were equivalent with respect to 5-hour GE, and not different from placebo. The GE delay with liraglutide 3.0 mg at 1 hour is unlikely to affect absorption of concomitantly administered oral drugs. The clinical relevance of the 1-hour GE delay and improved glycaemic parameters with 3.0 mg will be determined in the ongoing phase 3 programme in obese adults with and without T2D.

#### Effects of Liraglutide (Estimated Means)

	<b>Liraglutide 3.0 mg</b>	<b>Liraglutide 1.8 mg</b>	<b>Placebo</b>
Paracetamol (AUC <sub>0–300 min</sub> ) [min×µg/mL]	2252	2193	2424
Paracetamol (AUC <sub>0–60 min</sub> ) [min×µg/mL]	265*	299	343
FPG (mmol/L)	4.9*	4.9*	5.4
Glucose (iAUC <sub>0–300 min</sub> ) [min×mmol/L]	143**	192	192
Glucagon (iAUC <sub>0–300 min</sub> ) [min×pg/mL]	4866	4781*	6957
Insulin (iAUC <sub>0–60 min</sub> ) [min×mU/L]	3532*	3735*	4778
C-peptide (iAUC <sub>0–60 min</sub> ) [min×ng/mL]	208*	217*	263

AUC = area under the curve, i = incremental, FPG=fasting plasma glucose

\* p<0.05 vs. placebo.

\*\*p<0.05 vs. placebo and vs. liraglutide 1.8 mg.

Clinical Trial Registration Number: NCT00978393

Supported by: Novo Nordisk

## 710

**Bed time insulin versus GLP-1 analogues in patients with type 2 diabetes: comparison of short-term effects on resting energy expenditure**C. Gonzalez<sup>1</sup>, S. Salandini<sup>1</sup>, A. Pierreisnard<sup>1</sup>, M.-C. Beauvieux<sup>2</sup>, H. Gin<sup>1</sup>, V. Rigalleau<sup>1</sup>;<sup>1</sup>Diabetologie, <sup>2</sup>Biochimie, CHU Bordeaux, Pessac, France.

**Background and aims:** Poorly controlled type 2 diabetic patients (T2D) can be treated with GLP-1 analogues or bed-time insulin therapy with opposite influences on body weight. Beside the effects of GLP-1 analogues on energy intake, it is not known whether changes of Resting Energy Expenditure (REE) contribute to these distinct weight courses. We compared the early effects of both treatments on REE in T2D patients.

**Materials and methods:** Forty nine T2D patients (27 women, 22 men, age: 59±10 years, BMI: 32.8±4.8 kg/m<sup>2</sup>) poorly controlled despite maximal oral therapy (HbA1c: 9.9±1.4%), were included: 21 patients received GLP-1 analogues (exenatide\* 5µg twice-a-day or liraglutide\* 0.6 mg once-a-day); 28 received bed-time insulin analogue (Glargine\* or Detemir\* initial doses 0.2 UI/kg). REE was measured by indirect calorimetry before the first injection (Day0) and during two days (Day1 en 2) after initiating the treatment. Respiratory exchanges were monitored using a sensor MEDICS Vmax 29 N apparatus: VCO<sub>2</sub> and VO<sub>2</sub> were determined on 30 minutes intervals from 8 a.m to 8h30 p.m, before breakfast, and REE was calculated according to Weir's equation.

**Results:** On insulin treatment, REE decreased by -3.5% after the first injection (Day1: 1780±319 vs Day0: 1858±335 Kcal/24h, p<0.05) and by -7.5% after the second injection (Day2: 1713±237 Kcal/24h, P<0.005 vs Day1); REE was unchanged on GLP-1 treatment (Day1: 1829±287 Kcal/24h; Day2: 1861±309 Kcal/24h, NS). REE variations (Day2 vs Day0) were significantly different with these treatments: -162±241 Kcal/24h with Bed-time insulin vs -3 Kcal/24h with GLP-1 analogues; p=0.04. No difference in REE was found between Insulin analogues, nor between GLP-1 analogues.

**Conclusion:** REE decreased early on insulin therapy whereas it is not affected by GLP-1 analogues. These different effects on REE probably contribute to the opposite weight changes with these treatments. Our future work will be to assess if early changes in REE with bed-time insulin or GLP-1 analogues predict long-term weight evolution.

## 711

**Chronic exenatide infusion increases insulin sensitivity, decreases insulin secretion and stimulates islet cell proliferation in partially pancreatectomised baboons**A.M. Paez<sup>1</sup>, S. Kamath<sup>1</sup>, F. Casiraghi<sup>1</sup>, A. Davalli<sup>2</sup>, G. Abrahamian<sup>1</sup>, A. Ricotti<sup>1</sup>, S. La Rosa<sup>3</sup>, A. Commuzzie<sup>4</sup>, A. Marando<sup>3</sup>, G. Finzi<sup>3</sup>, E. Dick<sup>4</sup>, R. De Fronzo<sup>1</sup>, G. Halff<sup>1</sup>, F. Folli<sup>1</sup>;<sup>1</sup>UTHSCSA, San Antonio, USA, <sup>2</sup>SRI, Milano, Italy, <sup>3</sup>Circolo Hosp, Varese, Italy, <sup>4</sup>TBRI, San Antonio, USA.

**Background and aims:** Baboons are an interesting non-human primate model of insulin resistance and T2DM due to genetic and pathophysiologic similarities with humans. We studied the effects of a continuous IV exenatide (EXE) infusion after partial pancreatectomy (PP), on in-vivo glucose metabolism, α-β-δ cell mass and islet function in baboons.

**Materials and methods:** Two study groups: 1) EXE (n=12 F); 2) saline (SAL) (n=12 F). At baseline, all animals had a 2-step hyperglycemic clamp (1st step raise blood glucose +125mg/dL above fasting glucose; 2nd step: +225 mg/dL above fasting glucose) followed by an Arginine bolus (0.5g/kg). After completion of the hyperglycemic clamp, both groups underwent PP (the tail, ~30% of pancreas, was excised). Afterwards, a continuous EXE (0.014 µg/kg/h) or normal SAL infusion was started for 13 weeks. After 72 h EXE washout, clamps were repeated followed by euthanasia and removal of pancreas (head-body, ~70% of pancreas).

**Results:** Insulin sensitivity (M/I) increased by 41%, insulin secretion (ISR) decreased by 25% and β cell function=Disposition Index (ISR\*M/I) increased 50% after EXE, while they were unchanged in SAL. Morphometry by CAST, was used to quantify Islet's and immunostained α, β, δ cells' volume, expressed as % of total pancreas. PP SAL baboons had 42% decrease in islets in head-body vs tail (3.4±0.4 vs 6 ±0.6, p= 0.01), 43% decrease in β-cells (3.2±0.2 vs 5.5±0.5, p=0.003), 74% decrease in α- cells (0.6±0.2 vs 2.4±0.5, p=0.003) and 53% decrease in δ-cells (0.3±0.01 vs 0.7±0.1, p=0.02). After EXE there was only a 5% decrease in islets in head-body vs tail (6.2±0.9 vs 6.5±0.8, p= NS), 17% decrease in β cells (4.7±0.5 vs 5.6±0.8, p=NS), 21% decrease in α cells (1.6±0.5

vs 2.1±0.4, p=NS) and 15% increase in δ cells (0.8±0.01 vs 0.7±0.2, p=NS). EXE increased islet mass vs SAL in head-body (6.2±0.9 vs 3.4±0.4, p=0.01) suggesting that proliferation was involved. Proliferation and apoptosis were expressed as % of cells counted. MIB-1 staining, a cell proliferation marker, demonstrated proliferation after EXE, comparing baseline (tail) vs end of study (head-body) (0.07+ 0.03 vs 0.43+ 0.06, p=0.0001). There was also an increase in proliferation when comparing the EXE end of study vs SAL end of study (0.43+ 0.06 vs 0.07 + 0.04, p=0.0001). There were no differences between SAL basal vs end of study (0.07+ 0.04 vs 0.07 + 0.04, p=NS) or basal SAL vs basal EXE (0.07+ 0.04 vs 0.07 + 0.03, p=NS). Furthermore, EXE slightly attenuated post-PP apoptosis by M30 staining, tail vs head-body (1.58+ 0.58 vs 2.58 + 0.47, p=NS) as compared with SAL group which had significantly increased post-PP apoptosis, tail vs head-body (0.52 + 0.21 vs 1.75 + 0.55, p=0.05). Electron microscopy of SAL end of study pancreas demonstrated endoplasmic reticulum stress, degranulation and nuclear alterations consistent with apoptosis in β-cells. These changes were absent after EXE treatment.

**Conclusion:** Chronic IV EXE improves insulin sensitivity and disposition index, while decreasing glucose-arginine stimulated insulin secretion (a condition of β-cell rest). Furthermore, EXE stimulates proliferation and attenuates apoptosis of α-β-δ cells, thereby increasing islet of Langerhans mass in non-human primate pancreas, *in vivo*.

Supported by: NIH RO1 080148

## 712

**A high fat diet during pregnancy and lactation reverses the protection from obesity in Gpr<sup>-/-</sup> mice via altered hypothalamic and peripheral gene expression in offspring**F. Keyhani Nejad<sup>1,2</sup>, F. Isken<sup>1,2</sup>, M. Osterhoff<sup>1,2</sup>, A.F.H. Pfeiffer<sup>1,2</sup>, M. Kruse<sup>1</sup>;<sup>1</sup>Dept. of Clinical Nutrition, German Institute of Human Nutrition, Nuthetal, <sup>2</sup>Department for Endocrinology, Diabetes and Nutrition, Charité – University of Medicine, Berlin, Germany.

**Background and aims:** A high fat diet (HFD) consumed during gestation (IU) and lactation (L) predisposes offspring to develop obesity and insulin resistance in adulthood. Inhibition of Glucose -dependent insulintropic polypeptide (GIP) signaling in GIP receptor knock out mice (Gipr<sup>-/-</sup>) leads to protection from HFD induced obesity. Recently, we have shown that Gipr<sup>-/-</sup> mice which were exposed to HFD during IU/L and in early adulthood are no longer protected from diet induced obesity, had a decreased glucose tolerance, but showed reduced cumulative energy intake. Hypothalamic insulin sensitivity is necessary for balanced food intake and might also affect peripheral energy expenditure. We followed up on these observations and hypothesized that the potential programming effects of IU/L HF consumption on adiposity and food intake in Gipr<sup>-/-</sup> mice are due to altered hypothalamic insulin sensitivity and decreased peripheral fat oxidation.

**Materials and methods:** Female GIP receptor heterozygous (Gipr<sup>+/-</sup>) mice were fed either a HF (60%, fat) or control (C, 10% fat) diet for 2 weeks prior to mating with Gipr<sup>+/-</sup> male mice and during IU/L. Male Gipr<sup>-/-</sup> and wild type (WT) offspring were kept on normal chow for 22 weeks after weaning. At the age of 25 weeks, mice were fed the same HFD used during the IU/L for 20 weeks. This resulted into Gipr<sup>-/-</sup> which were exposed to either a C (KO Ciu-HF) or a HFD (KO HFiu-HF) during IU/L and a HFD later in adulthood. WT mice fed a control diet during IU/L and a HFD in adulthood served as controls (WT Ciu-HF). Body weight (BW) and food intake (FI) were recorded weekly. At the age of 45 weeks, gene expression levels of hypothalamic phosphatidylinositol 3-kinase (PI3K) and fatty acid oxidation in muscle were analyzed by quantitative RT-PCR and adipocyte sizes were analyzed using H&E staining histology.

**Results:** BW and FI between WT and Gipr<sup>-/-</sup> mice were not significantly different during the first 22 weeks. By re-exposure to HF at 25 weeks of age, body fat content significantly increased in KO HFiu-HF compared to KO Ciu-HF (22.6 ± 2.5 g vs. 19.5 ± 0.8 g, respectively; p<0.05). KO HFiu-HF had significantly increased adipocytes compared to KO Ciu-HF (p<0.005). Re-introduction to HF significantly reduced cumulative energy intake in KO HFiu-HF and WT Ciu-HF mice compared to KO Ciu-HF (37.7 ± 0.7 and 38.6 ± 1.6 vs. 43.5 ± 1.5, respectively; all p<0.01). In KO Ciu-HF hypothalamic gene expression of PI3K subunit p85α was 22% down regulated compared to WT Ciu-HF mice (p<0.01) and back up regulated 1.27 fold in KO HFiu-HF compared to KO Ciu-HF (P< 0.05). Gene expression levels of PPARα and PGC1α, the key enzymes of fatty acid oxidation, were massively increased in KO Ciu-HF compared to WT Ciu-HF mice (2.45-fold for PPARα and 1.85-fold for PGC1α, both P<0.01), but then down regulated 1.9-fold for PGC1α and 2.4-fold for PPARα in KO HFiu-HF compared to KO Ciu-HF (p<0.05) in muscle.



**Conclusion:** We conclude that consuming a high fat diet during pregnancy and lactation is able to reverse the favorable phenotype of inhibition of GIP signaling by decreasing central insulin sensitivity via up-regulation of regulatory subunit of PI3K and reducing the expression of key genes in fatty acid oxidation, which could account for the reduced body weight despite an increase in food intake.

Supported by: DFG, DDG

## 713

### Removal of L- and alpha cells improves oral glucose tolerance and accelerates diphtheria toxin induced weight loss in diabetic mice

J. Pedersen, S.M. Jørgensen, A. Plamboeck, J.A. Windeløv, J.J. Holst; Novo Nordisk Foundation Center for Basic Metabolic Research, Dept. Biomedical Sciences, University of Copenhagen, Denmark.

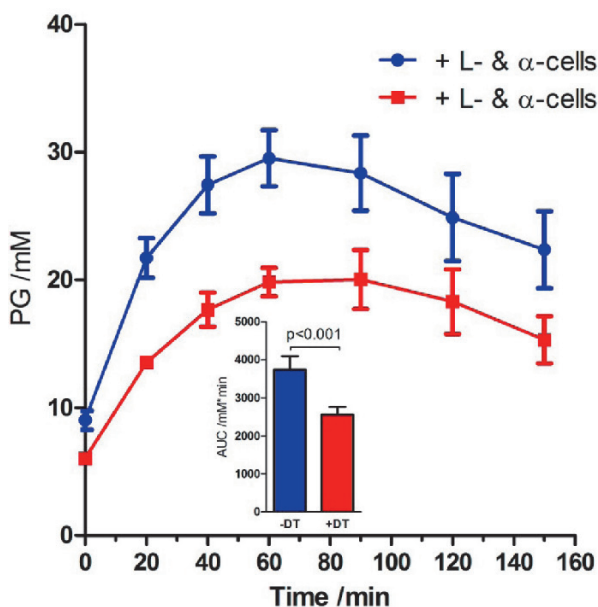
**Background and aims:** Proglucagon is a prohormone encoded by the *Glucagon* gene (*Gcg*) and several proglucagon derived peptides play a role in the regulation of metabolism. Pancreas specific post-translational processing produces glucagon, whereas intestinal and neuronal specific post-translational processing produces oxyntomodulin, glucagon-like peptide 1 and glucagon-like peptide 2. In both rodents and humans dysregulation of the secretion of proglucagon derived peptide hormones leads to impaired regulation of metabolism. The aim of the present study was to evaluate the consequences of removal of *Gcg* expressing cells in diabetic mice on glucose tolerance and bodyweight.

**Materials and methods:** The diphtheria toxin receptor *Gcg* cellular knock-out mouse (TgN(GCG.DTR)) allowed us to study the acute loss of proglucagon derived peptide hormones on glucose metabolism in mice metabolically stressed by high fat diet (60% of energy from fat) and single injection with low dose (100mg/kg) streptozotocin.

**Results:** Intraperitoneal injection with diphtheria toxin reduced immunoreactive glucagon content in tissue extract isolated from terminal ileum or pancreas with 50 and 10 fold respectively. Ablation of *Gcg* expressing cells in STZ injected mice placed on high fat diet improved oral glucose tolerance,  $AUC_{0-150min}$   $3744 \pm 350 \text{ mmol} \cdot \text{l}^{-1} \cdot \text{min}$  to  $2560 \pm 202 \text{ mmol} \cdot \text{l}^{-1} \cdot \text{min}$  ( $p < 0.001$ ). Removal of *Gcg* expressing cells, accelerated the weight loss following DT-injections  $1.21 \pm 0.19 \text{ g} \cdot \text{d}^{-1}$  vs  $0.65 \pm 0.10 \text{ g} \cdot \text{d}^{-1}$  ( $p < 0.02$ ), mostly explained by a loss of fat mass.

**Conclusion:** Our newly introduced genetically modified mouse allows the study of combined L- and  $\alpha$ -cell depletion in an already obese and diabetic mouse. Our findings underscore the diabetogenic effects of the unopposed  $\alpha$ -cells and antidiabetic effects of lack of  $\alpha$ -cells in models of type 2 diabetes. Surprisingly, our findings suggest an intact  $\alpha$ -cells mass is essential for body weight maintenance.

### Oral glucose tolerance TgN(GCG.DTR) +/- L- & $\alpha$ -cells



Supported by: Novo Nordisk Foundation

## 714

### Improvement of basal hyperglycaemia in diabetic GK rat after chronic treatment by liraglutide is associated with reduced alpha cell mass

J. Movassat<sup>1</sup>, A. Ilias<sup>1</sup>, S. Madani<sup>2</sup>, M. Alawieh<sup>1</sup>, D. Bailbé<sup>1</sup>, B. Portha<sup>1</sup>;

<sup>1</sup>University Paris Diderot, Paris, <sup>2</sup>Novo Nordisk, La Défense, France.

**Background and aims:** GLP-1 and its analogues have received much attention as a possible new treatment for type 2 diabetes. GLP-1 stimulates insulin secretion and biosynthesis and inhibits glucagon release. While GLP-1-induced beta cell proliferation and survival effects are well documented, there is a shortage of information on whether GLP-1 analogues modulate the rodent alpha-cell mass (ACM) under conditions of chronic T2DM. Our aim was to investigate, in the Goto-Kakizaki rat, a model of T2DM, the effects of a chronic treatment by the GLP1 analogue Liraglutide (Lira), 1/ on the beta-cell mass, the body weight (BW) gain, basal hyperglycemia and the glucose-induced insulin secretion in vivo; 2/ on basal plasma glucagon and on the (ACM) within the pancreas.

**Materials and methods:** Eight weeks old male GK rats were treated once daily (5:00 pm) subcutaneously at the dose of 0.2mg/kg BW, during 30 days (Lira group). Another group of rats was treated with saline solution (control group). The control group was pair-fed by adjusting their food intake at the equivalent amount of food taken by the rats of the Lira group. BW, food and water intake were measured daily. The basal glycemia was measured twice a week. Basal plasma insulin and glucagon were measured by RIA at days 0, 15 and 30 of the treatment. The glucose-induced insulin secretion in vivo was determined during an OGTT.

**Results:** Our results show that a 30 days Lira treatment improves the basal hyperglycemia of the diabetic GK rat (35% decrease). Importantly this was not associated with changes in basal or glucose-induced insulin secretion. However Lira treatment significantly reduced basal plasma glucagon (by 50 %) and surprisingly this was related with a significant decrease in the ACM (by 40%), whereas the beta cell mass was not affected. Experiments aimed to define the mechanisms for down-regulation of the ACM in response to Lira are in progress.

**Conclusion:** In conclusion, our data suggest that the beneficial effect of chronic Lira treatment on the hyperglycemic GK rat is due to the decrease of glucagon secretion which could in part be related to a decreased number of alpha-cells within the pancreas. Importantly, these Lira effects were observed independently of the Lira-induced diminution of food intake.

Supported by: Novo Nordisk

## 715

### GLP-1 analogue protect against inflammatory stress induced by diet and Acrp30 knockdown by inhibiting c-JNK

L. Xiaohu<sup>1,2</sup>, L. Li<sup>1,2</sup>, L. Zhang<sup>3</sup>, G. Yang<sup>3</sup>;

<sup>1</sup>College of Laboratory Medicine, <sup>2</sup>Key Laboratory of Laboratory Medical Diagnostics in Ministry of Education, <sup>3</sup>The Second Affiliated Hospital, Chongqing Medical University, China.

**Background and aims:** Non-alcoholic fatty liver disease (NAFLD), one of the most common chronic liver diseases, includes a spectrum ranging from simple steatosis to steatohepatitis and cirrhosis. Liraglutide, a Glucagon-like peptide-1 (GLP-1) analogue with 97% sequence identity to human GLP-1, increases insulin secretion and insulin sensitivity. Its effect on nonalcoholic fatty liver disease (NAFLD) remains poorly understood. Recently, it has been reported that GLP-1 directly reduced hepatic lipogenesis via the cAMP/AMPK pathway in DPP4-deficient rats [12]. More recently, some studies have implied that exendin-4 could prevent TNF- $\alpha$  induced JNK phosphorylation in beta cell [13] and decrease inflammatory gene expression in isolated human pancreatic islets. In this study, we examined whether liraglutide can protect against inflammatory stress by inhibiting activation of c-Jun N-terminal protein kinase (JNK).

**Materials and methods:** ApoE KO and adiponectin (Acrp30) knockdown mice fed a high-fat diet (HFD) were treated with liraglutide (10 mg/kg, twice daily) for 8 weeks. Hyperinsulinemic-euglycemic clamp studies were performed. Glucose rates of appearance (GRa) were determined with 3-[<sup>3</sup>H] glucose. Whole body GRa and glucose uptake (GR<sub>d</sub>) were calculated using the non-steady-state equation. Liver tissue was procured for histological examination, real-time RT-PCR and western blot analysis.

**Results:** The combination of HFD, Acrp30 knockdown and ApoE deficiency had additive effects on the development of insulin resistance (IR) and NAFLD. Administration of liraglutide prevented the development of HFD

and hypoadiponectinemia-induced IR and NAFLD in this model. Liraglutide also attenuated the expression of proinflammatory cytokines, including TNF- $\alpha$  and NF- $\kappa$ B, and the expression of two lipogenesis-related genes, Acetyl-CoA Carboxylase (ACC) and fatty acid synthase (FAS). These changes were accompanied by elevated plasma of Acrp30, increased Acrp30 mRNA, AMP Kinase phosphorylation, decreased mitogen-activated protein kinase 4 (MKK4) mRNA expression and JNK phosphorylation.

**Conclusion:** Our study demonstrated that liraglutide improved insulin sensitivity and low-grade inflammatory stress induced by HFD and hypoadiponectinemia and inhibited MKK4/JNK activation. The inhibitory effects of liraglutide on low-grade inflammatory stress and IR might be due to inhibition of JNK activation. Therefore, liraglutide may play a dual role in regulating insulin sensitivity and inflammatory responses via MKK4/JNK signaling. Thus, we believe that liraglutide or similar long-acting analogs may be useful to improve or prevent NAFLD. This may contribute to the beneficial effects of liraglutide on decreasing hepatic fat accumulation and improving insulin sensitivity in patients with type 2 diabetes.

*Supported by: National Natural Science Foundation of China (30971388, 30771037, 81070640)*

## 716

### Glucosensing responses of preproglucagon neurons in the nucleus tractus solitarius in vitro

S. Trapp<sup>1</sup>, K. Hisadome<sup>1</sup>, F. Reimann<sup>2</sup>, F.M. Gribble<sup>2</sup>;

<sup>1</sup>Surgery and Cancer, Imperial College London, London, <sup>2</sup>Cambridge Institute for Medical Research, University of Cambridge, UK.

**Background and aims:** Glucagon-Like Peptide-1 (GLP-1) is an incretin released from enteroendocrine L-cells postprandially and has glucoregulatory and satiety effects. GLP-1 is also produced as a neuropeptide by preproglucagon (PPG) neurons, found mainly in the nucleus tractus solitarius (NTS). We have previously shown that these neurons enhance their electrical activity in response to satiety peptides such as leptin and CCK, but it is currently unknown whether PPG neurons respond electrically to changes in local glucose availability, and thus might be involved in glucoprivic feeding responses.

**Materials and methods:** Adult transgenic mice expressing yellow fluorescent protein (YFP) under PPG promoter control were kept at a 12h light/dark cycle with ad libitum access to food and water. Coronal brainstem slices (200  $\mu$ m thick) containing the caudal NTS were obtained from these mice either at the beginning of the light period or at the beginning of the dark period, and maintained at 32°C in artificial cerebrospinal fluid (ACSF). Current- and voltage-clamp recordings were carried out on individual PPG neurons identified by their YFP fluorescence. ACSF Glucose concentrations were altered between 0.1 mM and 10 mM in order to elicit a glucosensing response.

**Results:** 48% of PPG cells from slices obtained during the light period were inhibited by glucose (GI): firing rate was  $3.9 \pm 1.5$  Hz,  $3.1 \pm 0.9$  Hz and  $2.0 \pm 0.7$  Hz ( $n=11$ ), in 0.1, 2 and 10 mM glucose, respectively. Another 11 cells showed no change in firing rate and 1 cell was excited by glucose (GE). In contrast, out of 29 PPG cells from slices obtained during the dark phase, 20 neurons were GE, with a firing rate of  $1.2 \pm 0.2$  Hz and  $1.9 \pm 0.3$  Hz in 1 mM and 10 mM glucose, respectively. 8 cells showed no change in firing rate and 1 cell was GI. Single-cell RT-PCR revealed that PPG neurons do not express glucokinase ( $n=18$ ) thus ruling out metabolic glucosensing equivalent to pancreatic  $\beta$ -cells. Finally, to test whether a Na<sup>+</sup>-dependent glucose transporter (SGLT) is involved in the GE response, PPG neurons were preincubated with the SGLT blocker phloridzin (10  $\mu$ M;  $n=7$ ) for 5 min and subsequently exposed to 10 mM glucose in the presence of phloridzin. This attenuated the increase in firing rate seen with 10 mM glucose by  $60 \pm 20\%$ .

**Conclusion:** A substantial proportion of NTS PPG neurons respond to changes in glucose availability. During the dark phase when mice are most active most PPG neurons show a GE response, consistent with a role of GLP-1 as satiety signal. This response appears to involve SGLT as previously found in GLP-1 releasing enteroendocrine cells. The circadian regulation of this response remains to be investigated. We hypothesise that PPG neurons are central to integrating peripheral satiety signals and brain energy status into a feeding response. Further studies should target these neurons *in vivo* to test this hypothesis.

*Supported by: EFSD/Lilly grant and MRC*

## 717

### RANTES reduces glucose-dependent GLP-1 secretion from intestinal L- cells

R. Pais, T. Zietek, H. Hauner, H. Daniel, T. Skurk;  
Technical University Munich, Freising, Germany.

**Background and aims:** Obesity is characterized by an increased production and secretion of pro-inflammatory factors such as acute phase proteins, cytokines and chemokines. The constitutively increased circulating levels of these factors are considered to contribute to a low grade inflammatory condition which may promote the co-morbidities of obesity like diabetes mellitus type 2. GLP-1 is a peptide hormone secreted from intestinal L-cells upon nutrient ingestion. It not only promotes insulin secretion from  $\beta$ -cells but also appears to protect from  $\beta$ -cell loss. GLP-1 secretory responses are impaired in obese and diabetic humans. RANTES is a small protein of 68 amino acids belonging to the C-C subfamily of chemokines. It promotes the recruitment and activation of inflammatory cells such as monocytes, lymphocytes, mast cells, and eosinophils and exerts its biological effects by binding to specific receptors that belong to the seven-transmembrane G-protein-coupled receptor (GPCR) family, namely CCR1, CCR3 and CCR5. RANTES is secreted from platelets, macrophages, eosinophils and fibroblasts and was only very recently described as a new adipokine. Its secretion positively correlates with adipocyte size and is gender specific and its serum levels and gene expression levels in white adipose tissue are higher in obese than in lean and higher in subjects with impaired glucose tolerance and type 2 diabetes. The aims of the present work were to 1) determine the presence of RANTES receptors, in the intestinal epithelium and in NCI-H716 cells, (2) to test the effects of recombinant RANTES on glucose stimulated GLP-1 secretion in vitro, and (3) to confirm the effects on glucose-dependent GLP-1 secretion in an in vivo model employing C57 BL/6 mice.

**Materials and methods:** To study this, we used the NCI-H716 cell line, a human intestinal GLP-1 secreting cell line and applied RANTES and measured GLP-1 output. We used techniques like RT-PCR, western blot and immunofluorescence to identify receptors for RANTES on the L-cell. We measured intracellular calcium and cAMP as downstream signaling pathways involved. Also we administered RANTES to mice and measured GLP-1 in plasma after a glucose bolus.

**Results and conclusion:** We demonstrate that RANTES reduces glucose stimulated GLP-1 secretion from in vitro cultures of human enteroendocrine cells with Met-RANTES (RANTES receptor antagonist) blocking this effect. We identified CCR1 at message and protein level in the NCI-H716 cells and mouse intestinal L-cells. RANTES reduced accumulation of cAMP in the NCI-H716 cells and also reduced glucose induced increase in intracellular calcium. Moreover, administration of RANTES in mice revealed reduced plasma GLP-1 and GLP-2 levels after an oral glucose load as well as impaired insulin secretion. These data suggests that elevated RANTES blood levels as found in obese individuals may cause the altered GLP-1 secretory response and identifies the RANTES-receptors as potential targets in diabetes therapy.  
*Supported by: DFG/GRK 1482*

## 718

### Development of <sup>125</sup>I-labeled exendin probe targeting GLP-1 receptors for detecting insulinoma cells

H. Fujimoto<sup>1</sup>, K. Toyoda<sup>1</sup>, H. Kimura<sup>2</sup>, Y. Ogawa<sup>2</sup>, H. Mstsuda<sup>2,3</sup>, M. Takagi<sup>3</sup>, A. Kon<sup>3</sup>, M. Ono<sup>2</sup>, H. Saji<sup>2</sup>, N. Inagaki<sup>1</sup>;

<sup>1</sup>Department of Diabetes and Clinical Nutrition, Graduate School of Medicine, Kyoto University, <sup>2</sup>Department of Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Science, Kyoto University, <sup>3</sup>Arkray, Inc, Kyoto, Japan.

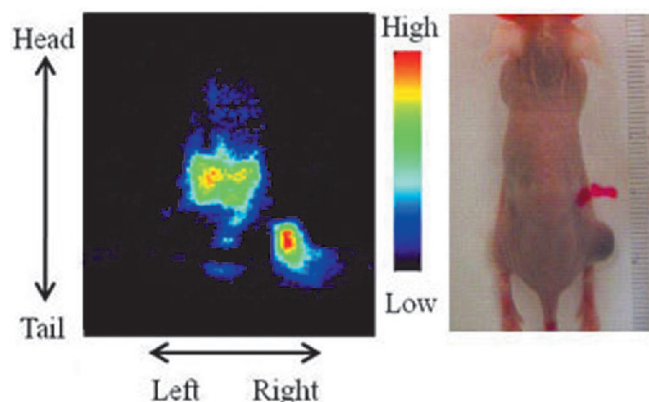
**Background and aims:** Among pancreatic neuroendocrine tumors, insulinoma is the most common tumor. About 90% of insulinomas is typically small size (<2 cm), therefore imaging methods need to be high sensitivity and specificity to the target for detecting such small tumors. It has been reported that insulinoma frequently expressed glucagon-like peptide-1 receptor (GLP-1R) with high density. Thus, a radiolabeled compound bound to GLP-1R has been expected to be the useful tool for imaging insulinoma. In this study, we designed and synthesized [<sup>125</sup>I]-N<sup>6</sup>-(3-[(<sup>125</sup>I]iodobenzoyl)lysine<sup>12</sup>]-Exendin(9-39)-NH<sub>2</sub> ([<sup>125</sup>I]IB12-Ex(9-39)) targeting GLP-1R on insulinoma cells, and evaluated its utility as a probe for SPECT imaging of insulinoma in the mouse model.

**Materials and methods:** For synthesizing [<sup>125</sup>I]IB12-Ex(9-39), the lysine residue of exendin(9-39) at 12 was labeled with [<sup>125</sup>I]. The binding affinity of

IB12-Ex(9-39) to human GLP-1R was evaluated by competitive binding assay using the prepared cell membranes. For assessment of the *in vivo* organ selectivity, biodistribution studies were conducted with INS-1-xenografted mice at 15, 30, 60, 120, and 360 min after [ $^{125}$ I]IB12-Ex(9-39) injection. SPECT imaging was performed 30min after injection of [ $^{125}$ I]IB12-Ex(9-39) into the INS-1-xenografted mice. In addition, stability of [ $^{125}$ I]IB12-Ex(9-39) *in vivo* was evaluated by analysis of the extracted tumor homogenate with reverse phase HPLC analysis.

**Results:** The results of binding assay analysis showed competitive inhibition of IB12-Ex(9-39) binding by non-radioactive GLP-1 and its IC<sub>50</sub> was 1.6 nM. The *in vivo* biodistribution of [ $^{125}$ I]IB-Ex(9-39) in INS-1-xenografted mice showed a high uptake in the tumor (12% dose/g at 6 h) and the ratio of radioactivity at 6 h in tumor-to-blood, tumor-to-muscle, tumor-to-pancreas were 30.0, 75.0, and 2.4, respectively. SPECT imaging studies revealed the remarkable accumulation of radioactivities at the site of INS-1 tumor (Arrow in the Figure). In addition, [ $^{125}$ I]IB12-Ex(9-39) showed high stability *in vivo*.

**Conclusion:** It was possible to detect the small tumor of insulinoma by using [ $^{125}$ I]IB12-Ex(9-39) as the probe for SPECT. Probes targeting GLP-1R would be useful for detecting such GLP-1R expressing tumors.



## PS 054 Beneficial effect of bariatric surgery and weight loss

719

### One year change in metabolic syndrome components in the Helping Evaluate Reduction in Obesity (HERO) study

J. Dixon<sup>1,2</sup>, T. Okerson<sup>3</sup>, C. Burk<sup>4</sup>, R. Shi<sup>3</sup>, D. Ng-Mak<sup>3</sup>, D. Globe<sup>3</sup>, N.A. Dreyer<sup>5</sup>;

<sup>1</sup>Baker IDI Heart and Diabetes Institute, Melbourne, Australia, <sup>2</sup>Monash University, Melbourne, Australia, <sup>3</sup>Allergan, Inc., Irvine, USA, <sup>4</sup>Health Outcomes Consultant, Laguna Beach, USA, <sup>5</sup>Outcome, a Quintiles Company, Cambridge, USA.

**Background and aims:** Few studies have assessed effect of weight loss following LAP-BAND® AP adjustable gastric banding (LAGB) on the change of Metabolic Syndrome (MeS) components in obese patients. No study has compared regional differences in components of MeS after banding.

**Materials and methods:** Subjects enrolled in the HERO 5-year prospective study of LAGB from the United States (US), Canada (CA), Australia (AU), and Europe (EU) and who had baseline (BL) as well as 1 Year (1 YR) data to determine MeS status, were included in this analysis (834/1,123). BL and 1 YR rates of MeS components were assessed and compared by regions using the NCEP ATP III MeS definition using Chi-square.

**Results:** Table 1 shows prevalence of MeS and its components at BL and 1 YR by regions. In general, reduced high density lipids (HDL) and raised blood pressure were the most prevalent components of MeS in addition to the waistline circumference that qualified patients for MeS at baseline and 1 YR. Of these, more patients were from the US and CA/AU than the EU at baseline ( $p < 0.05$ ) and 1 Yr ( $p > 0.05$ ). At 1 YR, median weight change was -16.5% (mean value was -16.9%) ( $p < 0.001$ ). Additionally, there was a decrease in MeS as well as a decrease in each of the components compared to BL ( $p < 0.05$ ) for the overall study group and for each region by 1 YR ( $p < 0.05$ ).

**Conclusion:** LAGB was associated with significant decreases in MeS and its components at 1 YR across all regions. Adjustable gastric banding may play a role in treatment of obese patients with MeS. Further long-term follow-up of these patients will help to determine durability of these results.

Table 1. Prevalence of Metabolic Syndrome and its Components in HERO study at baseline and Month 12 by Regions

	Metabolic Syndrome	Raised triglycerides or treatment for lipid abnormality	Reduced HDL or treatment for lipid abnormality	Raised blood pressure or treatment for hypertension	Raised fasting plasma glucose or treatment for Type 2 diabetes
Baseline					
Overall (N=834)	599 (71.8%)	361 (43.3%)	584 (70.0%)	609 (73.0%)	360 (43.2%)
US (N=539)	402 (74.6%)	228 (42.3%)	397 (73.7%)	394 (73.1%)	248 (46.0%)
CA/AU (N=125)	92 (73.6%)	66 (52.8%)	76 (60.8%)	108 (86.4%)	59 (47.2%)
EU (N=170)	105 (61.8%)	67 (39.4%)	111 (65.3%)	107 (62.9%)	53 (31.2%)
1 year*					
Overall (N=834)	356 (42.7%)	240 (28.8%)	355 (42.6%)	462 (55.4%)	221 (26.5%)
US (N=539)	232 (43.0%)	151 (28.0%)	241 (44.7%)	279 (51.8%)	145 (26.9%)
CA/AU (N=125)	59 (47.2%)	41 (32.8%)	51 (40.8%)	89 (71.2%)	37 (29.6%)
EU (N=170)	65 (38.2%)	48 (28.2%)	63 (37.1%)	94 (55.3%)	39 (22.9%)

\* $p < 0.05$  for all compared changes from baseline in MeS and each component for overall study and by regions.

Clinical Trial Registration Number: NCT00953173

720

### One year diabetes remission and improvement in the HERO (helping evaluate reduction in obesity) study

D.S. Ng-Mak<sup>1</sup>, M. Torre<sup>2</sup>, J. Dixon<sup>3,4</sup>, T. Okerson<sup>1</sup>, R. Shi<sup>1</sup>, D. Globe<sup>1</sup>;

<sup>1</sup>Allergan, Inc., Irvine, USA, <sup>2</sup>Allergan Medical, Goleta, USA, <sup>3</sup>Baker IDI Heart and Diabetes Institute, Melbourne, Australia, <sup>4</sup>Monash University, Melbourne, Australia.

**Background and aims:** Remission or improvement of type 2 diabetes (T2D) after gastric bypass surgery has been shown to be related to the duration of diabetes, the severity of disease, and amount of weight loss (or weight regain). However, few studies reported the factors related to diabetes remission after adjustable gastric banding (AGB). The overall objective of the HERO study is to examine the clinical effectiveness and safety of LAP-BAND® AP AGB over five years. This analysis seeks to identify factors associated with diabetes remission and improvement 1 year after AGB.



**Materials and methods:** The HERO study enrolled 1,123 subjects, 300 of whom reported either having a history of T2D or taking an anti-diabetic medication at baseline (BL); these were defined as having T2D at BL. The analysis sample included 199 subjects who provided complete BL and 1 year follow up data. Using the American Association of Clinical Endocrinologists (AACE) guidelines for T2D definition, diabetes remission was defined as HbA<sub>1c</sub> level of  $\leq 6.5\%$  and no anti-diabetic medication usage at 1 year. Diabetes improvement was defined as HbA<sub>1c</sub> level of  $\leq 6.5\%$ . Multivariate logistic regression was used to examine factors associated with remission and improvement at 1 year, controlling for region (US vs ex-US) and years of diabetes duration.

**Results:** Remission and improvement of T2D at 1 year were 39% and 64%, respectively. Baseline weight (kgs) was similar between those with remission (128.0), improvement (129.0) and no change (131.0). Those with remission or improvement had shorter diabetes duration in years than those without change (5.5 and 5.8 vs 8.6 respectively ( $p<.01$ )). At 1 year, both remission and improvement groups had significantly greater percent weight loss (%WL) than those without change (19.2%, 17.4% and 12.2%, respectively ( $p<.001$ )). Multivariate analysis showed that %WL (OR=1.11,  $p<.001$ ) was the most significant factor in predicting T2D remission at 1 year. Controlling for region (OR=2.6,  $p=.045$ ) and diabetes duration (OR=.89,  $p=.003$ ), subjects with 20%WL were 8.6 times more likely to have diabetes remission at 1 year. Similarly, multivariate analysis showed that %WL (OR=1.12,  $p<.001$ ) was the most significant factor in predicting T2D improvement at 1 year. Controlling for diabetes duration (OR=.86,  $p<.001$ ), subjects with 20%WL were 9.6 times more likely to have diabetes improvement at 1 year. No regional difference was observed in the prediction of diabetes improvement at 1 year.

**Conclusion:** AGB was associated with significant weight loss and resultant diabetes remission and improvement over 1 year. Our results are similar to the findings reported with RYGB, such that earlier intervention with bariatric surgery, and more specifically AGB, is more likely to result in diabetes remission. Future research is needed to investigate the interrelationship between beta-cell function, improved insulin sensitivity and AGB-facilitated weight loss on diabetes remission/improvement.

Clinical Trial Registration Number: NCT00953173

## 721

### Sustained reductions in weight and HbA<sub>1c</sub> with dapagliflozin: long-term results from phase III clinical studies in type 2 diabetes

C.J. Bailey<sup>1</sup>, J. Wilding<sup>2</sup>, M.A. Nauck<sup>3</sup>, E. Ferrannini<sup>4</sup>, A.A. Ptaszynska<sup>5</sup>, A.M. Apanovitch<sup>5</sup>, J. Sugg<sup>6</sup>, S.J. Parikh<sup>6</sup>

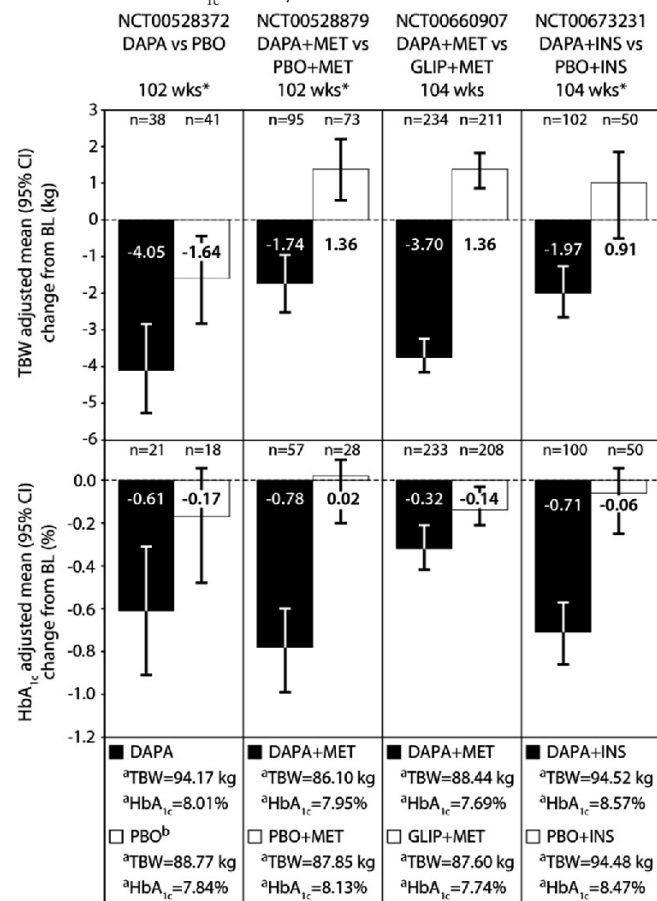
<sup>1</sup>Aston University, School of Life and Health Sciences, Birmingham, UK, <sup>2</sup>University Hospital Aintree, Liverpool, UK, <sup>3</sup>Diabeteszentrum Bad Lauterberg, Germany, <sup>4</sup>University of Pisa School of Medicine, Italy, <sup>5</sup>Bristol-Myers Squibb, Princeton, USA, <sup>6</sup>AstraZeneca, Wilmington, USA.

**Background and aims:** Dapagliflozin (DAPA), a selective inhibitor of sodium glucose co-transporter 2 (SGLT2), reduces plasma glucose independently of insulin secretion or action, by increasing urinary glucose excretion. In clinical trials up to 2 years, DAPA was associated with a reduction in total body weight (TBW), a key management goal in type 2 diabetes (T2D). This analysis reports TBW and HbA<sub>1c</sub> from four phase III clinical trials of DAPA, in predominantly overweight/obese patients with T2D, with different treatment backgrounds, and at different stages of disease.

**Materials and methods:** The effect of DAPA (10 mg/d) on TBW and HbA<sub>1c</sub> was assessed in four randomised, double-blind, placebo- (PBO) or comparator-controlled trials: DAPA monotherapy vs PBO (102 wks); DAPA add-on to metformin (MET) [DAPA+MET] vs PBO+MET (102 wks); DAPA+MET vs glipizide (GLIP)+MET (104 wks); DAPA+insulin (INS) [DAPA+INS] vs PBO+INS (104 wks).

**Results:** In all DAPA groups, the significant reductions in TBW and HbA<sub>1c</sub> observed at the end of the 24-wk short-term study were sustained for up to 2 years (Figure). At 102/104 wks, DAPA was associated with clinically meaningful reductions in TBW from baseline (BL) in each study (range: -1.74 to -4.05 kg). In contrast, over the same time period, weight gain from BL was observed in the PBO+MET (+1.36 kg), GLIP+MET (+1.36 kg) and PBO+INS (+0.91 kg) groups. PBO- or comparator-corrected adjusted mean reductions in TBW were in the range -2.41 to -5.06 kg. In addition, DAPA produced clinically meaningful reductions in HbA<sub>1c</sub> from BL (range: -0.32 to -0.78%), with PBO- or comparator-corrected adjusted mean reductions in HbA<sub>1c</sub> in the range -0.18 to -0.80%. In the DAPA groups, systolic blood pressure was either reduced or maintained in all studies (mean change from BL; range: -0.3 to -5.9 mmHg). Urinary glucose/creatinine ratio was elevated and maintained in all

studies, supporting the long-term efficacy of DAPA as an SGLT2 inhibitor. Adverse events were generally balanced across the groups. DAPA (10 mg/d) was not associated with a higher risk of hypoglycaemia. Events suggestive of genital and urinary tract infection were greater with DAPA. Infections occurred more often in the first 24 wks, were mostly mild to moderate in severity, managed by standard interventions, and without serious clinical consequences. **Conclusion:** In populations of overweight/obese patients with T2D, treated across the typical course of the disease, DAPA administered as monotherapy or in combination with MET or INS was associated with sustained decreases in both TBW and HbA<sub>1c</sub> over 2 years.



<sup>a</sup>Excludes data after start of rescue medication or INS up-titration.

<sup>a</sup>mean at baseline; <sup>b</sup>+MET after 24 wks. Error bars = 95% confidence interval (CI).

Clinical Trial Registration Number: NCT00528372; NCT00528879; NCT00660907; NCT00673231

Supported by: BMS and AZ

## 722

### Adipose tissue TCF7L2 splicing is regulated by weight loss and associates with glucose and fatty acid metabolism

D. Kaminska<sup>1,2</sup>, T. Kuulasmaa<sup>3</sup>, S. Venesmaa<sup>3</sup>, P. Käkälä<sup>3</sup>, M. Vaitinen<sup>1</sup>, L. Pulkkinen<sup>1</sup>, M. Pääkkönen<sup>3</sup>, H. Gylling<sup>1</sup>, M. Laakso<sup>2</sup>, J. Pihlajamäki<sup>1,2</sup>

<sup>1</sup>Institute of Public Health and Clinical Nutrition, <sup>2</sup>Department of Medicine,

<sup>3</sup>Department of Surgery, University of Eastern Finland and Kuopio University Hospital, Finland.

**Background and aims:** We have recently published, that expression of several genes regulating alternative splicing is reduced in liver and muscle of obese individuals. TCF7L2 consists of 18 exons and is subject to extensive alternative splicing in different human tissues. We investigated the effects of obesity surgery induced weight loss on TCF7L2 alternative splicing in adipose tissue and liver. Furthermore, we determined the association of TCF7L2 splicing with the levels of plasma glucose and serum free fatty acids (FFAs) in three independent studies (n=216).

**Materials and methods:** Alternative splicing in adipose tissue was investigated using PCR-capillary electrophoresis on samples extracted from subcu-

taneous and visceral adipose tissue of 95 participants of the ongoing Kuopio Obesity Surgery Study (KOBS). The association between alternatively spliced variants and metabolism was analyzed also in two independent study groups: a total of 49 men from the population-based METSIM study (Metabolic Syndrome in Men) and 113 non-diabetic individuals from the EUGENE2 study (European Network on Functional Genomics of Type 2 Diabetes).

**Results:** Expression of the short mRNA variant, lacking exons 12, 13 and 13a, decreased after weight loss in subcutaneous fat ( $n=46$ ;  $p=0.0001$ ) and liver ( $n=11$ ;  $p=0.008$ ). Furthermore, the variant was more common in subcutaneous fat of subjects with type 2 diabetes than in subjects with normal glucose tolerance both in obese individuals ( $n=54$ ;  $p=0.002$ ) and in a population-based sample ( $n=49$ ;  $p=0.031$ ). Additionally, there was a positive correlation between this variant and the level of fasting glucose in non-diabetic individuals ( $n=113$ ;  $p=0.026$ ). This association between TCF7L2 splicing and plasma glucose was independent of the TCF7L2 genotype. Finally, this variant associated with high levels of serum FFAs during hyperinsulinemia ( $p=0.0009$ ) suggesting impaired insulin action in adipose tissue, whereas no association with insulin secretion or insulin-stimulated whole body glucose uptake was observed.

**Conclusion:** Our study shows that the short TCF7L2 mRNA variant in subcutaneous fat is regulated by a weight loss and is associated with hyperglycemia and impaired insulin action in adipose tissue.

Supported by: Academy of Finland and Emil Aaltonen Foundation

## 723

### High insulin levels should be used for selection of patients with morbid obesity for bariatric surgery to achieve the best reduction of cardiovascular events

H.P. Kopp<sup>1</sup>, J.M. Brix<sup>1</sup>, G.H. Scherthaner<sup>2</sup>, G. Scherthaner<sup>1</sup>;

<sup>1</sup>Department of Medicine I, Rudolfstiftung Hospital, <sup>2</sup>Department of Medicine II, Medical University of Vienna, Austria.

**Background and aims:** Worldwide a huge number of patients undergo bariatric surgery, but the selection criteria for patients to achieve the best benefits from surgery are unclear. In the on-going debate for identification of possible indicators, preferably to be measured pre-surgery, we investigated the association of baseline Insulin levels with a detailed cardiovascular risk factor profile (CVRFP) up in one of the largest consecutive cohorts of patients with morbid obesity (MO) available.

**Materials and methods:** In total 1134 patients with MO (mean BMI:  $45.1 \pm 6.6$  kg/m<sup>2</sup>; mean age:  $39 \pm 12$  years; 79.4% females) were studied. Blood samples were taken from all patients to measure kidney, inflammatory parameters, fasting blood glucose, HbA1c and Insulin levels as well as lipids. Thus a CVRFP was established. Patients were divided according to their Baseline Insulin level into tertiles (0–18 µU/ml; 18,01–28.39 µU/ml; >28.4 µU/ml) into groups of 378 patients each. Differences were tested by using ANOVA as appropriate.

**Results:** Patients within the highest insulin tertile (see table) were significantly younger ( $p<0.001$ ), had a higher BMI ( $p<0.001$ ) and had the highest CVRFP: Higher levels for systolic and diastolic blood pressure, triglycerides and blood glucose levels as well as lower levels HDL cholesterol, and the lowest LDL-cholesterol ( $p<0.001$ ). 6.8% of patients in the lowest insulin tertile where suffering Type 2 Diabetes, whereas in the second insulin tertile 11.3% of patients and in the highest insulin tertile 21.5% of patients had Type 2 diabetes.

**Conclusion:** Our study indicates that MO patients with Insulin levels in the highest tertile at baseline have the worst cardiovascular risk factor profile and might thus be the best candidates for bariatric surgery. Thus the patients with the highest unmet need of cardiovascular mortality and events reduction would be selected for the procedure which has been become limited to high demands and costs, recently.

Table

	First Tertile	Second Tertile	Third Tertile	p-value
Systolic Blood Pressure, mmHg	139±17	143±17	145±16	$p<0.001$
Diastolic Blood Pressure, mmHg	88±10	91±11	92±12	$p<0.001$
C-reactive Protein, pg/ml	0.86±0.66	1.01±0.64	0.95±0.66	$p=0.007$
BMI, kg/m <sup>2</sup>	43±6	45±6	47±7	$p<0.001$
Waist circumference, cm	127±14	134±14	139±15	$p<0.001$
Fasting, blood Glucose, mg/dl	92±28	95±25	101±27	$p<0.001$
HbA1c, %	5.7±0.8	5.9±0.8	6.0±1.0	$p<0.001$
eGFR (MDRD), ml/min/1.73m <sup>2</sup>	90.8±21.3	91.8±21.6	89.4±22.7	$p=0.3$
HDL-cholesterol, mg/dl	53±13	48±14	44±12	$p<0.001$
Triglycerides, mg/dl	48±14	157±85	179±100	$p<0.001$
LDL-cholesterol, mg/dl	126±36	120±34	114±32	$p<0.001$

## 724

### Differences in glucose, triglycerides, insulin resistance, and appetite suppression after Roux-en-Y gastric bypass and sleeve gastrectomy for morbid obesity

A. Kokkinos<sup>1</sup>, K. Alexiadou<sup>1</sup>, C. Liaskos<sup>1</sup>, I. Balla<sup>1</sup>, G. Argyrakopoulou<sup>1</sup>,

T. Angelopoulos<sup>1</sup>, M. Fasoulaki<sup>1</sup>, D. Perrea<sup>2</sup>, A. Alexandrou<sup>3</sup>,

N. Katsilambros<sup>1</sup>, T. Diamantis<sup>3</sup>, N. Tentolouris<sup>1</sup>;

<sup>1</sup>First Department of Propaedeutic Medicine, <sup>2</sup>Laboratory of Experimental Surgery and Surgical Research “NS Christeas”, <sup>3</sup>First Department of Surgery, Athens University Medical School, Greece.

**Background and aims:** Bariatric surgery is currently the most effective method for weight loss in morbid obesity, with variable, however, effects on glucose and lipid metabolism. The aim of this study was to compare the impact of two bariatric procedures on glucose, triglyceride and insulin serum levels, as well as insulin resistance and the subjective perception of hunger and satiety.

**Materials and methods:** Twenty three obese patients were recruited. Eleven underwent Roux-en-Y Gastric Bypass (RYGB, BMI:  $48.1 \pm 6.0$  kg/m<sup>2</sup>, age:  $38.1 \pm 8.0$  years) and 12 sleeve gastrectomy (SG, BMI:  $50.1 \pm 7.4$  kg/m<sup>2</sup>, age:  $39.8 \pm 7.5$  years,  $p$  vs RYGB=NS). Patients visited our department preoperatively as well as 3 and 6 months postoperatively. Blood samples were collected after an overnight fast and every 30 minutes after consumption of a 450 kcal (200 ml ice cream) test meal until 180 minutes postprandially. They also completed a visual analog scale questionnaire for the subjective rating of hunger and satiety every 30 minutes. Differences in glucose, triglycerides, insulin, hunger and satiety were examined in terms of area under the curve divided by time (AUC) using the trapezoid rule. Insulin resistance was estimated with the HOMA-IR index (Homeostatic Model Assessment-Insulin Resistance).

**Results:** Patients in both groups had no preoperative differences in any of the above variables and experienced significant ( $p<0.001$ ) and comparable weight loss both 3 and 6 months postoperatively (BMI for RYGB at 6 months:  $35.2 \pm 4.9$  vs SG:  $37.2 \pm 6.2$  kg/m<sup>2</sup>,  $p=NS$ ). A significant reduction in insulin resistance at 3 (RYGB:  $p=0.026$ , SG:  $p=0.027$ ) and 6 months (RYGB:  $p=0.01$ , SG:  $p=0.001$ ), glucose at 6 months (RYGB:  $p=0.007$ , SG:  $p=0.022$ ) and insulin at 3 and 6 months ( $p<0.01$ ) in both groups was observed. Moreover, the RYGB group displayed a significant reduction in triglyceride levels at 6 months ( $p=0.033$ ) and a reduction in the perception of hunger at 3 ( $p=0.016$ ) and 6 months ( $p=0.044$ ). Patients in either group did not experience significant differences in satiety perception. Comparing the two procedures, there was a significant difference in triglyceride concentrations 3 (AUC RYGB:  $107 \pm 26$  vs SG:  $139 \pm 26$  mg/dl,  $p=0.013$ ) and 6 months (AUC RYGB:  $86 \pm 26$  vs SG:  $135 \pm 33$  mg/dl,  $p=0.002$ ) after surgery.

**Conclusion:** Both RYGB and SG lead to significant and comparable weight loss, as well as decreases in glucose, insulin, and insulin resistance. Gastric bypass favors an improved postprandial triglyceride response and a blunted sense of hunger.

## 725

**Arterial stiffness decreases after bariatric surgery in patients with morbid obesity**R. Tirado<sup>1</sup>, G. Llauro<sup>1</sup>, M. Villaplana<sup>1</sup>, P. Rebas<sup>2</sup>, A. Luna<sup>2</sup>, B. Pons<sup>1</sup>, J.M. González-Clemente<sup>1</sup>, A. Caixàs<sup>1</sup><sup>1</sup>Endocrinology and Nutrition Department, <sup>2</sup>Surgery Department, Hospital de Sabadell. Corporació Sanitaria Parc Taulí. Sabadell. Institut Universitari Parc Taulí- UAB Universitat Autònoma de Barcelona, Sabadell, Spain.

**Background and aims:** Increased arterial stiffness is independently associated with high risk for cardiovascular morbidity and mortality. Morbidly obese subjects have increased arterial stiffness, compared with healthy subjects, that decreases after weight loss. The aim of this study is to measure the arterial stiffness in morbidly obese subjects, before and after bariatric surgery, and to study its relationship with anthropometric parameters, insulinresistance index and plasma lipids.

**Materials and methods:** Forty-four patients have been studied, 39 women and 5 men, (age  $43.05 \pm 7.96$  years, BMI  $44.9 \pm 4.45$  Kg/m<sup>2</sup>). Forty-eight percent had hypertension, 34% type 2 diabetes and 39% dislipidaemia. The arterial stiffness was assessed by applanation tonometry on the radial artery (Sphygmocor<sup>®</sup> versión 7.0 AtCor Medical, Sidney, Australia). The variables obtained were: the augmentation index adjusted for heart rate (IAx@75), central systolic pressure (CSP) and central pulse pressure. Also, BMI, waist circumference, % body fat by bioelectrical impedance, (TANITA), HOMA insulinresistance index (HOMA) and plasma lipid levels were measured. All measurements were done at baseline and one year after bariatric surgery. In a subgroup of patients (n=21), carotid-femoral pulse wave velocity (PWV) was also measured before and until 2 years after surgery. For the statistical analysis SPSS-PC-plus version 19 was used.

**Results:** At 12 months after surgery, all patients showed a decrease in BMI ( $44.9 \pm 4.46$  vs  $29.4 \pm 3.54$  Kg/m<sup>2</sup>), % body fat ( $49.9 \pm 4.11$  vs  $33.8 \pm 7.15$  %), waist circumference ( $131.3 \pm 12.9$  vs  $102.4 \pm 11.0$  cm) and HOMA ( $4.61 \pm 2.60$  vs  $1.3 \pm 0.83$ ), and an improvement of lipid parameters,  $p < 0.001$  in all of them. CSP and IAx@75 also decreased at 12 months ( $110.4 \pm 18.3$  vs  $124.8 \pm 15.9$  mmHg and  $21.13 \pm 12.6$  vs  $25.07 \pm 11.0$  %,  $p < 0.005$ , respectively). The reduction in IAx@75 and CSP, after bariatric surgery correlated only with the reduction in the percentage of body fat ( $b = 0.544$ ,  $p < 0.01$   $y = 0.357$ ,  $p = 0.028$ ). PWV also decreased at 2 years after surgery ( $7.52 \pm 1.56$  vs  $6.84 \pm 1.14$  m/s,  $p = 0.004$ ) but no correlations were found with any of the variables.

**Conclusion:** Morbidly obese patients show a decrease in arterial stiffness after bariatric surgery related to the decrease in body fat.

*Supported by: CIR 2011/017 Fundació Parc Taulí*

## 726

**Insulin secretion and sensitivity after bariatric surgery: laparoscopic gastric banding vs biliopancreatic diversion**J. Vrbikova<sup>1</sup>, T. Grimmichova<sup>1</sup>, K. Dvorakova<sup>1</sup>, J. Vcelak<sup>1</sup>, O. Bradnova<sup>1</sup>, T. Halkova<sup>1</sup>, M. Fried<sup>2</sup>, P. Sramkova<sup>2</sup>, K. Dolezalova<sup>2</sup>, R. Bilek<sup>1</sup>, M. Hill<sup>1</sup>, V. Hainer<sup>1</sup>, P. Mc Ternan<sup>3</sup>, I. Kyrou<sup>3</sup>, S. Kumar<sup>3</sup><sup>1</sup>Institute of Endocrinology, Prague, Czech Republic, <sup>2</sup>OB Klinika, Prague, Czech Republic, <sup>3</sup>University Hospital Coventry and Warwickshire, WISDEM, Warwick Medical School, University of Warwick, Coventry, UK.

**Background and aims:** Mechanisms by which bariatric surgery improves diabetes remain still elusive. We compare the effects of laparoscopic gastric banding (LAGB) and biliopancreatic diversion (BPD) on insulin sensitivity and insulin secretion.

**Materials and methods:** Women with type 2 diabetes mellitus (T2 DM) and obesity referred to bariatric surgery (LAGB, n=11; age  $54.4 \pm 9.5$  years, BMI  $44.7 \pm 6.8$  kg/m<sup>2</sup> T2DM lasting for  $4.5 \pm 4$  years; BPD, n = 12, age  $49.7 \pm 6$  years, BMI  $48 \pm 7.6$  kg/m<sup>2</sup>, T2DM lasting for  $5.7 \pm 6.7$  years) were examined by euglycaemic clamp and mixed meal test with modelling of insulin secretion before, and 1 and 6 months after the operation. Results are given as median (lower quartil; upper quartil). Statistics: ANOVA

**Results:** BMI improved more after BPD (from  $44.3(42.3; 55.4)$  to  $41(38.6; 52.4)$  and to  $35.9(34.5; 39.2)$  vs in LAGB  $44.2(36.1; 50.4)$  to  $42.1(33.48.1)$  and  $38.4(29.5; 42.6)$  kg/m<sup>2</sup>;  $p = 0.001$ ). HbA<sub>1c</sub> decreased significantly (in BPD from  $52.5(47.5; 58.8)$  to  $45.5(44.3; 48.5)$  and to  $39.5(38.4; 45.8)$ , in LAGB from  $51(47.5; 55)$  to  $45(44.0; 49.0)$  and to  $43.5(40.8; 46.3)$  mmol/mol;  $p = 0.001$ ) and fasting blood glucose improved (in BPD from  $7.85(7.1; 10.6)$  to  $5.85(5.6; 6.38)$  and to  $5.85(5.45; 7.1)$ ; in LAGB from  $8.1(7.9; 11.1)$  to  $6.4(5.9; 7.4)$  and to  $6.5(5.9; 7.25)$  mmol/l;  $p = 0.001$ ) after both types of operations. Insulin sensitivity

expressed as glucose disposal per kg of fat free mass (FFM) improved similarly after both LAGB and BPD (in BPD from  $20.2(13.6; 32.4)$  to  $30(21.6; 46.5)$  and to  $33.1(30.6; 35.8)$ ; in LAGB from  $16.4(14.8; 26.1)$  to  $27.3(26.2; 33.1)$  and to  $37.3(25.9; 40.6)$   $\mu$ mol/kg FFM;  $p = 0.02$ ), whereas insulin secretion early after meal (ISR 15min) at 1 month postoperatively decreased in BPD (from  $3.79(2.85; 3.87)$  to  $2.31(2.19; 2.96)$  pmol/kg min;  $p = 0.05$ ) but did not change after LAGB ( $4.21(3.8; 4.34)$  vs.  $3.8(3.4; 4.12)$  pmol/kg min). Glucose dependent insulinotropic peptide (GIP) levels decreased significantly after BPD but not after LAGB (30min from  $480(422; 595)$  to  $263(196; 310)$  and to  $260(244; 325)$  pg/ml;  $p = 0.001$ ; 60min from  $328(309; 658)$  to  $195(171; 258)$  and to  $268(220; 358)$  pg/ml;  $p = 0.001$ ; 120min from  $371(231; 537)$  to  $222(155; 271)$  and to  $265(155; 479)$  pg/ml;  $p = 0.001$ ; 180min from  $269(225; 367)$  to  $183(142; 238)$  and to  $225(155; 340)$  pg/ml;  $p = 0.01$ ). Glucagon-like peptide 1 increased after BPD but no significant change after LAGB was seen in 60min (from  $128(110; 165)$  to  $161(128; 194)$  and to  $175(167; 193)$  pg/ml;  $p = 0.05$ ). GLP-1 at 180min increased after BPD from  $131(123; 150)$  to  $189(146; 243)$  and to  $174(153; 208)$  pg/ml;  $p = 0.05$ ) but decreased (from  $167(112; 183)$  to  $156(112; 176)$  and to  $150(135; 296)$   $p = 0.05$ ) after LAGB. GIP30 after BPD correlates with ISR 15 but not with insulin sensitivity ( $r = 0.496$ ,  $p = 0.004$ ).

**Conclusion:** Both LAGB and BPD led to the improvement in insulin sensitivity. Insulin secretion did not change after LAGB. However, after BPD, early insulin secretion decreased and was correlated with decreased GIP levels.

*Supported by: EFSD New Horizons grant*

## 727

**Decreased micro-particel associated tissue factor activity in morbid obese patients after bariatric surgery**L. Ay<sup>1</sup>, J.M. Brix<sup>1</sup>, J. Thaler<sup>2</sup>, G. Scherthaner<sup>3</sup>, C. Ay<sup>2</sup>, I. Pabinger<sup>2</sup>, G. Scherthaner<sup>1</sup><sup>1</sup>Department of Medicine I, Rudolfstiftung Hospital, Vienna, <sup>2</sup>Division of Haematology and Haemostaseology, Department of Medicine I, Medical University of Vienna, <sup>3</sup>Department of Medicine II, Medical University of Vienna, Austria.

**Background and aims:** Tissue factor (TF) plays a crucial role in the initiation of blood coagulation. Active circulating TF was detected on small negatively charged membrane vesicles, so called MPs, which are released upon cell activation and apoptosis from variety of cells. Increased number and/or procoagulant activity of microparticles (MPs) have been reported in morbid obese patients and may mediate the thrombotic propensity. Our objective was to determine the effect of bariatric surgery on the MP-associated tissue factor (TF) activity in morbid obese patients.

**Materials and methods:** MP-TF activity was measured with a TF-dependent factor Xa generation assay. We investigated MP-TF activity in morbid obese patients (n= 74) that were included in our study. Seventy-four MO patients (mean age:  $42 (\pm 11)$  years; 61 female) were investigated before and 2 years after bariatric surgery. Within this period the body mass index decreased from (mean  $\pm$  SD)  $45 \pm 10$  to  $30 \pm 7$  ( $p < 0.001$ ).

**Results:** A significant improvement of metabolic parameters was observed after weight loss (Table 1) and of c-reactive protein from preoperatively  $1.1 \pm 1.1$  mg/dL to  $0.4 \pm 0.6$  mg/dL. Postoperatively the median (25th-75th percentile) MP-TF activity decreased significantly from  $0.120(0.180-0.480)$  pg/mL to  $0.022(0.000-0.279)$  pg/mL ( $p = 0.002$ ). There was a statistically significant correlation between the preoperatively MP-TF activity and c-reactive protein ( $r = 0.3$ ;  $p = 0.02$ ).

**Conclusion:** In conclusion, we could demonstrate for the first time a significant decrease of the MP-TF activity using a chromogenic assay after weight loss in morbid obese patients.

Table 1

	Pre-Surgery	Post-Surgery	p-value
HbA1c, %	$6.0 \pm 1.0$	$5.4 \pm 0.5$	$p < 0.001$
total cholesterol, mg/dl	$204 \pm 34$	$179 \pm 35$	$p < 0.001$
HDL cholesterol, mg/dl	$50 \pm 12$	$58 \pm 19$	$p < 0.001$
LDL cholesterol, mg/dl	$122 \pm 32$	$101 \pm 31$	$p < 0.001$
Triglycerides, mg/dl	$130 \pm 21$	$90 \pm 20$	$p < 0.001$



## 728

### Inhibition of gut peptide responses to food ingestion modulates the brain's response to food ingestion after Roux-en-Y gastric bypass: a FDG-PET neuroimaging study

K.F. Hunt<sup>1</sup>, J.T. Dunn<sup>2</sup>, P.K. Marsden<sup>2</sup>, L.J. Reed<sup>3</sup>, A.G. Patel<sup>4</sup>, S.A. Amiel<sup>1</sup>;

<sup>1</sup>Diabetes Research Group, Diabetes and Nutritional Sciences Division, King's College London School of Medicine, <sup>2</sup>Division of Imaging Sciences, King's College London School of Medicine, <sup>3</sup>Neuropsychopharmacology Unit, Imperial College London, <sup>4</sup>King's College Hospital, London, UK.

**Background and aims:** Roux-en-Y gastric bypass (RYGB) causes sustained weight loss but the mechanisms are not fully understood. Modulation of central control of appetite by exaggerated gut peptide responses to eating may contribute. Inhibition of gut peptide responses, using somatostatin, decreases fullness and increases food intake after RYGB.

**Aim:** To investigate the effect of inhibiting gut peptide responses to food ingestion on the brain responses to eating in people who have lost weight post RYGB, using [18F]-fluorodeoxyglucose positron emission tomography (FDG-PET) functional neuroimaging.

**Materials and methods:** Seven people 19±13 months post RYGB (weight loss 31.3±9.6%; BMI 34.2±4.2 kg/m<sup>2</sup>; HOMA2-IR 0.68±0.22) underwent 4 FDG-PET scanning visits after overnight fasting in random order in a 2x2 factorial design: with intravenous (IV) somatostatin (0.1 mcg/kg/min) and IV basal insulin replacement (3.6 mU/m<sup>2</sup>/min) or with IV saline (placebo); and consuming 400 kcal meal before scanning (FED) or remaining fasted (FASTED). Satiety was assessed on 0–100 visual analogue scales (VAS); blood was sampled; regional brain FDG uptake, a surrogate for neuronal activation, was measured from 15 to 70 minutes post meal. Two non-operated groups, one obese (n=8; BMI 34.2±2.7 kg/m<sup>2</sup>; HOMA2-IR 0.56±0.2) and one normal weight (n=11; BMI 22.2±1.5 kg/m<sup>2</sup>; HOMA2-IR 0.53±0.16) have been studied in a similar protocol.

**Results:** With placebo, the FED (compared to FASTED) state post-RYGB was associated with decreased (voxelwise  $p<0.001$ , cluster size threshold 100 voxels) FDG uptake in regions involved in interoception (insular); reward (ventral striatum & globus pallidus); cognitive control (dorsolateral frontal cortex, DLFC, & anterior cingulate cortex, ACC); and resting state network (precuneus & parietal lobule), whereas in non-operated subjects FED (vs FASTED) was associated with increased FDG uptake in regions involved in reward (ventral striatum & globus pallidus) and, in the normal weight group only, a small decrease in FDG uptake in regions involved in cognitive control (DLFC & ACC). Post RYGB somatostatin, vs placebo, inhibited post-meal increases in insulin (+0.5±1.3 vs +35.5±25.5 mU/l), glucagon-like peptide-1 (+0.1±0.5 vs +16.1±10.7 pM) and peptide YY (-1.0±0.6 vs +79.7±25.7 pg/ml) and decreased fullness at 10 minutes (49.8 vs 74.8,  $p=0.049$ ). Post RYGB somatostatin, vs placebo, also attenuated the effect of food ingestion in insular, ventral striatum, globus pallidus, DLFC, ACC, precuneus and parietal lobule ( $p<0.05$ ) towards the patterns seen in the non-operated groups.

**Conclusion:** Increased fullness on eating after RYGB is associated with reversal of the brain's response to food ingestion in regions involved in reward, and replication of the normal deactivation in regions involved in cognitive control. These changes in the central responses to food ingestion, which may associate with negative rather than positive experiences of eating and improved executive control, may contribute to the weight loss seen after RYGB. The response to somatostatin is consistent with altered gut peptide response to eating mediating a major part of this effect.

Clinical Trial Registration Number: 02683156

Supported by: The Diabetes Foundation

## PS 055 Incretin based therapies

## 729

### Exenatide plus metformin compared to metformin alone on beta cell function in type 2 diabetic patients

P. Maffioli<sup>1</sup>, I.G. Franzetti<sup>2</sup>, F. Querci<sup>3</sup>, A. Carbone<sup>4</sup>, L. Ciccirelli<sup>5</sup>, M. Piccinni<sup>6</sup>, E. Fogari<sup>1</sup>, M.A. Ferraro<sup>7</sup>, G. Derosa<sup>1</sup>;

<sup>1</sup>Internal Medicine and Therapeutics, University of Pavia, IRCCS Policlinico S.Matteo, Pavia, <sup>2</sup>Metabolic Unit, Regional Hospital, Varese, <sup>3</sup>Diabetes Care Unit, Ospedale Pesenti Fenaroli, Bergamo, <sup>4</sup>Internal Medicine, Hospital Center of Diabetes, Lodi, <sup>5</sup>Internal Medicine, RSA Villa Mafalda, Pavia, <sup>6</sup>Internal Medicine, Fondazione Ospedale della Carità, Cremona, <sup>7</sup>Diabetologia, PST Gallico, Reggio Calabria, Italy.

**Background and aims:** In previously reported studies, exenatide proved to be effective in reducing insulin resistance and increasing  $\beta$ -cell function, giving also a decrease of body weight and inflammatory state. The aim of this study was to quantify how much exenatide added to metformin improves  $\beta$ -cell function, and to evaluate the impact on glycemic control, insulin resistance, and inflammation compared to metformin alone.

**Materials and methods:** 174 Caucasian type 2 diabetic patients, naïve and with poor glycaemic control, were instructed to take metformin for 8±2 months until a mean dosage of 2500±500 mg/day, then they were randomly assigned to take exenatide (5 µg twice a day for the first 4 weeks, 10 µg twice a day thereafter) or placebo volume equivalent for 12 months. We evaluated at 3, 6, 9, and 12 months: body mass index (BMI), glycemic control, fasting plasma insulin (FPI), homeostasis model assessment insulin resistance index (HOMA-IR), homeostasis model assessment  $\beta$ -cell function index (HOMA- $\beta$ ), fasting plasma proinsulin (FPPr), proinsulin/fasting plasma insulin ratio (Pr/FPI ratio), C-peptide, glucagon, adiponectin (ADN), high sensitivity-C reactive protein (Hs-CRP), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Before, and after 12 months since the addition of exenatide, patients underwent a combined euglycaemic hyperinsulinemic and hyperglycaemic clamp, with subsequent arginine stimulation, to assess insulin sensitivity and insulin secretion. Continuous variables were evaluated using analysis of variance (ANOVA) tests. Intervention effects were adjusted for the presence of potential confounding variables using analysis of covariance (ANCOVA). ANOVA was also used to assess variables' significance within and between groups.

**Results:** Exenatide+metformin gave a greater decrease of body weight and BMI ( $p<0.01$  for both), glycaemic control ( $p<0.05$ ), FPI ( $p<0.05$ ), FPPr ( $p<0.05$ ), FPPr/FPI ratio ( $p<0.05$ ), HOMA-IR ( $p<0.05$ ), and glucagon ( $p<0.05$ ) values and a greater increase of C-peptide levels ( $p<0.05$ ), HOMA- $\beta$  ( $p<0.05$ ) and ADN ( $p<0.05$ ) compared to placebo+metformin. Exenatide+metformin, but not placebo+metformin, also decreased TNF- $\alpha$  and Hs-CRP ( $p<0.05$  compared to baseline for both, not significant vs placebo+metformin). Exenatide+metformin treatment gave a greater increase of M value (+34%), and Disposition Index (+55%) compared to placebo+metformin; 1<sup>st</sup> (+21%) and 2<sup>nd</sup> phase (+34%) C-peptide response to glucose and C-peptide response to arginine (+25%) were also improved by exenatide+metformin treatment, but not by placebo+metformin.

**Conclusion:** Exenatide confirmed its efficacy on glycaemic control, and its positive effective in protecting  $\beta$ -cell and in reducing inflammation.

## 730

### GLP-1 agonists and insulin in obese insulin-treated patients with type 2 diabetes: combined treatment or substitution? Weight and metabolic benefits

F. Bertoin, A. Paoli, D. Ancelle, B. Decoudier, M. Francois, C. Lukas-Croisier, E. Bertin, M. Crouzet, P. Moysset, A.-C. Hécart, D. Malgrange, B. Delemer; Reims University Hospital, France.

**Background:** GLP-1 agonists have pleiotropic effects. They particularly increase satiety and decrease glucagon secretion which might be interesting in insulin-treated type 2 diabetic patients even more when they are obese.

**Aims:** To assess and to compare the weight and metabolic benefits obtained with two different therapeutic strategies: Insulin in combination with GLP-1 agonist or switch insulin to GLP-1 agonist (to respect the product license in France).

**Materials and methods:** French monocentric retrospective study from 2008 to 2010 with a cohort of 148 insulin-treated type 2 diabetic patients.

**Results:** 101 patients had association (group 1); 47 patients had switch (group 2). Baseline characteristics were similar in both groups except insulin daily dose which was significantly lower in group 2 (0.97 vs 0.66 units/day). Mean age: 59.3 vs 57.8 years old; mean BMI: 38.9 vs 39.3 kg/m<sup>2</sup>; diabetes duration: 19.2 vs 16.3 years; insulin was begun after a ten-year evolution; mean HbA1c: 8.6%. The addition of GLP-1 agonists to insulin enables a decrease of HbA1c about -0.84 % at 6 months and about -0.34% after one year, a weight loss of 5 Kg, a 9% reduction of the basal insulin dose and a withdrawal of rapid insulin in 30% of cases. Substitution leads to a better weight benefit ( $p=0.007$ ) but without any glycemic interest which becomes worse in 45% (21/47) of the patients. No result after a 6 month- treatment predicts the absence of response afterwards and should lead to GLP-1 agonist discontinuation.

**Conclusion:** Adding GLP-1- analogs and maintain insulin seems to be the safest strategy. This association results in a satisfying weight loss with a slight improvement of HbA1c and a reduction of the global daily insulin dose.

## 731

### Add-on treatment of GLP-1 based therapy in patients with type 2 diabetes treated with insulin: an observational study

W.T. Zandee<sup>1</sup>, M.A. Sleddering<sup>2</sup>, I. Jazet<sup>2</sup>, A.H. Bootsma<sup>1</sup>, P.H.L. Geelhoed-Duijvestijn<sup>1</sup>

<sup>1</sup>Internal Medicine, Medical Center Haaglanden, The Hague, <sup>2</sup>Internal Medicine, Leiden University Medical Center, Netherlands.

**Background and aims:** In the Netherlands GLP-1 based therapy is reimbursed to treat type 2 diabetes (DM2) not in control with a combination of metformin and SU-derivatives and a BMI>35. However, patients already on insulin might also benefit from this therapy by losing weight and lowering insulin dose. A 5% or more decrease in weight is known to have significant metabolic effects. Thus far, only few results have been published on the combination with insulin in daily practice. Aim of this study is to evaluate the results of GLP-based therapy in addition to insulin, treated “off-label” in our outpatient diabetes clinic.

**Methods:** Data were collected from all patients in our Medical Center using insulin, who were started on either liraglutide or exenatide. HbA1c, weight and insulin dose were collected at baseline, at six months and one year after the start and/or at last follow-up. Values were compared using a mixed model for repeated measurements. Categorical variables were analyzed with the McNemar’s test for paired proportions. A backward conditional linear regression analysis was performed to identify independent determinants of the decrease in HbA1c, body weight and total daily dose (TDD) of insulin at 6 months follow-up.

**Results:** 150 patients were included. Mean age was 56 years and the mean follow-up was 10.4 months. 33 patients stopped within 3 months of therapy most of them due to side effects or an increase in glucose values. Baseline HbA1c was 9.32% and 8.82% and 8.86% at 6 and 12 months resp. ( $P<0.05$ ). The average weight decreased from 105.3kg to 99.2kg after 12 months ( $P<0.01$ ). At 6 months 59% of patients lost more than 5% of their body weight. Average insulin dose decreased from 73.8IU to 18.7 IU after 12 months ( $P<0.01$ ). Insulin was discontinued in 56% of patients. At baseline 5.0% of patients reached a HbA1c <7.0% and after 6 months this number increased to 16.5% ( $P<0.01$ ). HbA1c decrease at 6 months was predicted by insulin dose at baseline (negative) and the decrease in weight (positive) ( $R=0.40$ ,  $R^2=0.16$ ,  $F=9.77$ ). Decrease in weight was predicted by decrease in TDD of insulin (positive) and HbA1c at baseline (negative), ( $R=0.52$ ,  $R^2=0.27$ ,  $F=19.37$ ,  $P<0.001$ ). Decrease of insulin dose was predicted by HbA1c at baseline (negative), the decrease in weight after 6 months (positive) and the addition of an SU-derivate (positive), ( $R=0.50$ ,  $R^2=0.26$ ,  $F=12.64$ ,  $P<0.001$ ).

**Conclusion:** Our results show that GLP-1 based therapy can successfully be used to treat obese DM2 not in control on insulin and results, if tolerated, in a 10% increase of patients reaching treatment goals.

## 732

### Safety and efficacy of liraglutide in type 2 diabetes: post marketing surveillance data from India

S.K. Wangnoo<sup>1</sup>, A. Mithal<sup>1</sup>, S. Kumar<sup>2</sup>, K.D. Modi<sup>3</sup>, P. Shamanna<sup>4</sup>, R. Shetty<sup>5</sup>; <sup>1</sup>Dept. of Endocrinology, Indraprastha Apollo Hospital, New Delhi, <sup>2</sup>Dept. of Endocrinology & Metabolism, Sir Ganga Ram Hospital, New Delhi, <sup>3</sup>Dept. of Endocrinology, Shanti Niketan, Hyderabad, <sup>4</sup>Bangalore Diabetes Centre, Kalyan Nagar, Bangalore, <sup>5</sup>Novo Nordisk India Pvt. Ltd, Bangalore, India.

**Background and aim:** Liraglutide is recently approved and available in India. Here we present an interim analysis of a post marketing surveillance (PMS) (LEAD-IN) study of liraglutide in Indian subjects with type 2 diabetes mellitus (T2DM). The objective of this study was to assess the safety and efficacy of liraglutide in real-life situations in India.

**Material and methods:** LEAD-IN is an ongoing multicenter, open-label, PMS study for 52 weeks. Subjects with T2DM  $\geq 18$  years; with or without prior treatment with anti-diabetic drugs and considered clinically suitable for liraglutide therapy were enrolled. Subjects are planned to be evaluated at baseline, after 13 and 26 weeks of therapy for safety and efficacy. Additionally it is planned to evaluate subjects for the next 26 weeks (total 52 weeks) for safety. During the study clinically required concomitant medications were allowed. Here we present data of an interim analysis of 13 weeks.

**Results:** Total 1416 subjects (M: 809; F: 607) were enrolled in the study and 1322 completed 13 weeks follow up. The mean age was 46.8 years and mean duration of diabetes was 7.18 years. Total 16 subjects reported adverse events (excluding hypoglycaemia) the most common adverse events were nausea (N=7), vomiting (N=9) and back pain (N=1). At baseline, 104 (7.3%) and 5 (0.4%) subjects reported minor and major hypoglycaemia preceding 4 weeks which was reduced to 64 (4.8%) to 0(0%) after 13 weeks of liraglutide therapy. No serious adverse event was noted. Furthermore significant improvement in serum creatinine and lipid profile was noticed. Table 1 shows reduction in glycaemic and non-glycaemic parameters. At 13 weeks, change in HbA1c, FPG and PPG was  $-1.03 \pm 0.86$  ( $-5.8;3.1$ );  $-29.7 \pm 33.43$  ( $-231;126$ ) and  $-58.1 \pm 52.40$  ( $-270; 135$ ) respectively.

**Conclusion:** This interim analysis of the open label, observational LEAD-IN study demonstrates the favourable safety of liraglutide in Indian subjects of T2DM in real-life situation. Additionally significant improvement in glycaemic control was noticed.

**Table 1: Reduction in Glycaemic and Non-glycaemic Parameters**

Parameter	Baseline (Mean $\pm$ SD)	After therapy (Mean $\pm$ SD)	p value
HbA1C (%)	8.77 $\pm$ 1.26	7.76 $\pm$ 0.93	<0.0001
FPG (mg/dL)	170.00 $\pm$ 47.97	140.10 $\pm$ 35.05	<0.0001
PPG* (mg/dL)	246.80 $\pm$ 61.23	190.30 $\pm$ 46.11	<0.0001
Body weight (kg)	92.54 $\pm$ 14.62	88.71 $\pm$ 13.57	<0.0001
BMI (kg/m <sup>2</sup> )	34.38 $\pm$ 5.49	32.97 $\pm$ 5.21	<0.0001
SBP (mm of Hg)	134.40 $\pm$ 15.26	126.50 $\pm$ 9.44	<0.0001
DBP (mm of Hg)	85.50 $\pm$ 8.79	82.30 $\pm$ 5.51	<0.0001
Serum Creatinine (mg/dL)	1.02 $\pm$ 0.70	0.90 $\pm$ 0.42	<0.0001
Urine Albumin (mg/dL)	12.11 $\pm$ 15.69	10.85 $\pm$ 13.35	0.166
Total Cholesterol (mg/dL)	187.30 $\pm$ 42.64	172.78 $\pm$ 32.99	<0.0001
HDL (mg/dL)	43.62 $\pm$ 12.07	47.21 $\pm$ 16.31	<0.0001
LDL (mg/dL)	109.16 $\pm$ 36.75	102.34 $\pm$ 29.40	<0.0001
Triglyceride (mg/dL)	152.02 $\pm$ 66.87	130.79 $\pm$ 52.72	<0.0001

\* post breakfast; paired t-test was applied,  $p < 0.005$ : statistically significant.

Clinical Trial Registration Number: CTRI/2010/091/001280

Supported by: Novo Nordisk India Pvt Ltd, LEAD-IN PMS study

## 733

**One-year effects of liraglutide on pancreatic beta cell function and glycaemic control in Japanese type 1 diabetes with residual insulin secretion**

Y. Hamamoto, K. Mori, S. Honjo, Y. Kawasaki, K. Fujimoto, H. Tatsuoka, A. Matsuoka, Y. Wada, H. Ikeda, H. Koshiyama;  
Center for Diabetes and Endocrinology, Kitano Hospital, Osaka, Japan.

**Background and aims:** Since GLP-1 receptor agonists have been reported to be protective against  $\beta$ -cell destruction, they are expected to be beneficial for glycemic control in patients with type 1 diabetes (T1D) with residual  $\beta$ -cell function. The aim of this study was to investigate the effect of liraglutide, a long-acting GLP-1 receptor agonist, on pancreatic  $\beta$ -cell function and glycaemic control in Japanese patients with T1D with residual insulin secretion.

**Materials and methods:** In this open-labeled, randomized, parallel-group study, a total of ten subjects with type T1D (age  $48.5 \pm 12.1$  yrs, BMI  $22.8 \pm 1.8$ ; mean  $\pm$  SD) who were positive for GADAb or IA-2Ab and had been treated with insulin more than one year with residual insulin secretion which was defined by C-peptide (CPR) more than 0.3 ng/ml were included. Group A was administered liraglutide 0.9 mg in addition to ongoing insulin therapy for 52 weeks, and insulin group B continued the ongoing insulin injection. Changes in HbA1c, blood glucose (BG) with self-monitoring of blood glucose (SMBG), postprandial plasma CPR levels, body weight, dose of insulin injection per day, and adverse events (AE) were investigated. Pancreatic  $\beta$ -cell function was evaluated by glucagon- and arginine-stimulation test which was performed before and 52 weeks after, and CPR index which were calculated by the following formula [CPR/Blood Glucose (BG)].

**Results:** In group A, HbA1c value tended to decrease from  $7.6 \pm 1.4\%$  to  $7.3 \pm 1.5\%$  at 52 weeks with decreased daily insulin dose from  $40.8 \pm 20.6$  to  $33.8 \pm 17.4$  units although it did not reach statistical significance ( $p=0.39$ ). On the other hand, in group B, HbA1c remained unchanged from  $7.7 \pm 0.7$  to  $7.8 \pm 0.9\%$  with slightly increased insulin dose from  $37.7 \pm 12.2$  to  $40.2 \pm 14.2$  units. It is notable that insulin dose was reduced in 80% of group A whereas none in group B could reduce it. Averaged incremental CPR value in arginine-stimulated test and CPR secretion in response to glucagon-stimulation showed a slight decrease in both groups but there was no statistical significance between the groups. However, in subjects with obvious improvement of glycemic control, liraglutide increased casual CPR levels and CPR response to arginine-stimulation suggesting that the responses to liraglutide was varied in the individual cases. The CPR index calculated with postprandial CPR and BG tended to increase in group A but not in group B. No AE including severe hypoglycemia was observed in both groups.

**Conclusion:** These data suggest that addition of liraglutide on ongoing insulin therapy has a potential to improve and stabilize BG with reduction of daily insulin dose. The major effect of liraglutide was likely to be exhibited through extra- $\beta$ -cell effects such as suppression of glucagon secretion rather than an increase of insulin secretion. However, a beneficial effect on  $\beta$ -cell function was suggested, indicating that longer period observation might detect the beneficial effect. Further investigation with increased number of subjects and longer period of observation will be needed, and currently the study is still ongoing.

Clinical Trial Registration Number: UMIN000006919

## 734

**Microalbuminuria is an independent risk factor for nausea caused by glucagon-like peptide-1 receptor agonists**

H. Umematsu, R. Suzuki, M. Awazawa, Y. Hada, Y. Izumida, N. Shojima, Y. Okazaki, M. Fujishiro, Y. Iizuka, H. Sakoda, M. Ohsugi, K. Hara, T. Yamauchi, K. Ueki, T. Kadowaki;  
Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, Japan.

**Background and aims:** Nausea is one of the most common adverse reactions caused by treatment with glucagon-like peptide-1 receptor agonists (GLP-1 RAs) for type 2 diabetes. Although this symptom often leads to withdrawal of the treatment, it is yet to be clarified whether we can predict high-risk patients in advance. The objective of this study was to investigate the association of clinical parameters and incidence of nausea among patients with type 2 diabetes during treatment with GLP-1 RAs.

**Materials and methods:** We conducted a case-control study using the medical records of adult type 2 diabetes patients who started treatment with GLP-1 RAs between September 2010 and January 2012 during hospitalization at our

hospital. The number of patients who experienced nausea was retrospectively counted. Microvascular complications and clinical parameters were evaluated in nausea group and non-nausea group. Microalbuminuria was defined as a spot urine albumin-to creatinine-ratio (ACR) between 30 and 300 mg/g. Severity of diabetic retinopathy was evaluated by ophthalmologists. Diabetic neuropathy was diagnosed based on impairment of vibration sense or diminished deep tendon reflexes. Two-group comparisons were made using  $\chi^2$  tests and student t tests. To assess significant predictors of nausea, we performed multivariate analysis in a stepwise multiple logistic-regression model.

**Results:** GLP-1 RAs (0.3 mg once-daily liraglutide or 5  $\mu$ g twice-daily exenatide, as standard initial dose in Japan) were administered to 66 adult patients (36 males and 30 females; age  $59 \pm 12$  yrs; duration of type 2 diabetes  $11.7 \pm 7.8$  yrs; HbA1c  $9.0 \pm 1.6\%$ ; BMI  $30.5 \pm 6.9$  kg/m<sup>2</sup>). At baseline, the patients were treated with diet therapy alone (1%), oral hypoglycemic agents (38%), or insulin (61%). All cases were reviewed and none were excluded. Nausea was observed in 26 patients (39%). The discontinuation rate was 9%. Between the nausea and non-nausea groups, urine ACR (215 mg/g vs 84.3 mg/g), HbA1c (8.4% vs 9.3%), and estimated GFR (67.8 ml/min/1.73 m<sup>2</sup> vs 84.0 ml/min/1.73 m<sup>2</sup>) were significantly different ( $P=0.0085$ ,  $P=0.014$ ,  $P=0.017$ , respectively). Gender, age, BMI, duration of type 2 diabetes, waist circumferences, and the brands of GLP-1 RAs were not different statistically. According to multivariate logistic regression analysis, presence of microalbuminuria was significantly associated with nausea ( $P=0.0058$ ), even after adjustment for the brands of GLP-1 RAs, HbA1c, estimated GFR, diabetic retinopathy, diabetic neuropathy, and hypertension. In albuminuria (microalbuminuria or proteinuria) group ( $n=36$ ), the incidence rate of nausea was significantly increased (OR 2.50 [95% CI 1.10–5.73]  $P=0.048$ ). However, between microalbuminuria group ( $n=22$ ) and proteinuria group ( $n=14$ ), no significant difference was observed (OR 1.29 [95% CI 0.73–2.28]). Data are given as mean  $\pm$  SD.

**Conclusion:** Microalbuminuria is linked with an increased risk of nausea due to GLP-1 RAs. Gastrointestinal symptoms should be carefully monitored when GLP-1 RAs are administered to patients with albuminuria.

## 735

**A possible way to improve the glycaemic control and the beta cell function during three years: the triple therapy**

G. Derosa<sup>1</sup>, P.D. Ragonesi<sup>2</sup>, A.F.G. Cicero<sup>3</sup>, I.G. Franzetti<sup>4</sup>, F. Querci<sup>5</sup>, A. Carbone<sup>6</sup>, M.N. Piccinni<sup>7</sup>, A. D'Angelo<sup>1</sup>, E. Fogari<sup>1</sup>, P. Maffioli<sup>1</sup>;  
<sup>1</sup>Internal Medicine and Therapeutics, University of Pavia, IRCCS Policlinico S. Matteo, Pavia, <sup>2</sup>Diabetes Care Unit, S. Carlo Hospital, Milan, <sup>3</sup>"G. Descovich" Atherosclerosis Study Center, University of Bologna, <sup>4</sup>Metabolic Unit, Regional Hospital, Varese, <sup>5</sup>Diabetes Care Unit, Ospedale Pesenti Fenaroli, Bergamo, <sup>6</sup>Diabetes Care, Hospital Center of Diabetes, Lodi, <sup>7</sup>Internal Medicine, Fondazione Ospedale della Carità, Cremona, Italy.

**Background and aims:** When metformin monotherapy is not enough to reach an adequate glycaemic control, combination therapy is required. The aim of this study was to evaluate the effects of a triple therapy of metformin + pioglitazone + sitagliptin or metformin + pioglitazone + glibenclamide compared to metformin monotherapy or a double combination of metformin + pioglitazone on glycaemic control, insulin resistance, and  $\beta$ -cell function in type 2 diabetic patients.

**Materials and methods:** During the two years run-in period, 476 patients were instructed to take metformin and pioglitazone until a mean dosage of 1850  $\pm$  15 mg/day. At the end of the run-in period, 445 patients were randomised to add glibenclamide 5 mg three times a day or sitagliptin 100 mg once a day to metformin 2200 mg/day + pioglitazone 30 mg/day for one year in a double blind design. We evaluated at the baseline, and after 6, 12, 18, 24, 30 and 36 months: glycated hemoglobin (HbA<sub>1c</sub>), fasting plasma insulin (FPI), HOMA-IR, HOMA- $\beta$ , fasting plasma proinsulin (FPPR), C-peptide. At the baseline and every six months, patients underwent a combined euglycaemic hyperinsulinemic and hyperglycaemic clamp. Continuous variables were evaluated using analysis of variance (ANOVA) tests. Intervention effects were adjusted for the presence of potential confounding variables using analysis of covariance (ANCOVA). For all statistical analyses,  $p < 0.05$  was considered statistically significant.

**Results:** Both the triple therapies were more effective in reducing HbA<sub>1c</sub> compared to metformin monotherapy ( $p < 0.001$ ), and dual therapy metformin + pioglitazone ( $p < 0.02$ ). FPI, and HOMA-IR were significantly increased by triple therapy with glibenclamide ( $p < 0.05$  vs metformin, and  $p < 0.05$  vs metformin + pioglitazone), and decreased by the one with sitagliptin ( $p < 0.05$  vs glibenclamide). While sitagliptin did not change HOMA- $\beta$ , it was significantly increased by glibenclamide ( $p < 0.0001$  vs metformin, and  $p < 0.0001$  vs



pioglitazone). FPPr was not influenced by triple therapy with glibenclamide, while it was decreased by the one with sitagliptin ( $p<0.01$  vs metformin, and  $p<0.05$  vs glibenclamide). Both glibenclamide and sitagliptin triple therapies increased C-peptide compared to baseline ( $p<0.05$  for glibenclamide, and  $p<0.02$  for sitagliptin). Regarding clamp derived measures of  $\beta$ -cell function, triple therapy with sitagliptin better improved all the measures compared to the glibenclamide one ( $p<0.05$  for all), and also compared with metformin monotherapy ( $p<0.01$ ), and metformin + pioglitazone ( $p<0.05$ ).

**Conclusion:** Dual combination therapy is more effective than monotherapy in improving glycaemic control; furthermore, when metformin and pioglitazone combination is not enough, sitagliptin should be preferred to glibenclamide as addition to the already ongoing therapy, for its better protection of  $\beta$ -cell secretion, and its neutral effect on body weight.

## 736

### The gut hormone GLP-1 improves the postprandial (PP) glycaemia in type 2 diabetes mellitus by insulin, glucagon, and gastric emptying

B. Aulinger, A. Bedorf, G. Kutscherauer, B. Göke, J. Schirra;

Department of Internal Medicine II, Campus Großhadern, University of Munich, Germany.

**Background and aims:** Gut born GLP-1 decreases PP glycemia in healthy subjects by stimulation of insulin and reduction of glucagon. GLP-1 inhibits gastric motility. The insulinotropic action contributes to the incretin effect, i.e. the difference between the PP and the isoglycemic fasting insulin response. A defect of this enteroinsular axis has been suggested in T2DM, the function of GLP-1 is, however, unknown. We examined the antidiabetic effects of GLP-1 in T2DM using the GLP-1R antagonist exendin(9-39) (Ex-9) with and without stimulation of the endogenous GLP-1 by the DPP-4 inhibitor Sitagliptin.

**Materials and methods:** Double-blind, placebo-controlled, four-arm-cross-over study in 24 patients with T2DM ( $61\pm7$  y, HbA1c  $6.2\pm0.2$ , BMI  $27.7\pm0.9$ ). On four days separated by a wash-out period of at least one week blood glucose, plasma insulin and glucagon concentrations, and gastric emptying (13C acetate breath test) were measured during the four hours after ingestion of an OGTT (75g). In a randomized fashion, oral treatment with placebo or 100 mg Sitagliptin was combined with an intravenous infusion of saline or Ex-9 at 900 pmol/kg/min. The dose of Ex-9 used here blocks the insulinotropic effect of GLP-1 in human by at least 95%. On four further days blood glucose excursions obtained during the OGTTs were mimicked by intravenous glucose (isoglycemic clamp) for calculation of the incretin effect.

**Results:** see table. Compared to saline iv Ex-9 increased PP blood glucose ( $P=0.004$ ), decreased both plasma insulin ( $P<0.001$ ) and the incretin effect ( $P=0.012$ ), and increased plasma glucagon ( $P=0.005$ ) excursions with and without Sitagliptin. Sitagliptin increased PP plasma GLP-1 ( $P<0.001$  vs placebo po) and lowered PP blood glucose ( $P<0.001$  vs placebo po) not only without but also with Ex-9. In spite of the lower PP glycemia, Sitagliptin increased PP plasma insulin ( $P<0.001$ ), and this again was not dependent on background infusion. Sitagliptin enhanced the incretin effect compared to placebo ( $P=0.043$ ) which was reduced but eliminated under Ex-9. During the first 120min Sitagliptin lowered PP plasma glucagon with saline iv ( $P=0.045$ ), but not with Ex-9 iv ( $P=0.221$ ). Ex-9 accelerated and Sitagliptin delayed gastric emptying, but Sitagliptin had no effect on gastric emptying during Ex-9.

**Conclusion:** Endogenous GLP-1 lowers PP glycemia after an OGTT in T2DM by stimulation of insulin, inhibition of glucagon and delay of gastric emptying. Acute DPP-4 inhibition by Sitagliptin improves glucose tolerance by enhancing endogenous GLP-1 action. However, Sitagliptin decreases PP glycemia by stimulation of insulin even independent of GLP-1.

Blood glucose, plasma hormones and gastric emptying after an OGTT in T2DM

	Placebo po + saline iv	Sitagliptin po + saline iv	Placebo po + Ex-9 iv	Sitagliptin po + Ex-9 iv
<b>Glucose</b> (g/dl•240 min)	18.5 $\pm$ 1.1	15.1 $\pm$ 1.1 *	20.1 $\pm$ 1.2#	18.4 $\pm$ 1.4 *#
<b>Insulin</b> (mU/ml•240 min)	7.8 $\pm$ 1.4	9.0 $\pm$ 1.4 *	6.2 $\pm$ 1.0 #	7.6 $\pm$ 1.2 *#
<b>Incretin effect</b> (C-peptide) ( $\mu$ g/ml•240 min)	3.4 $\pm$ 1.0	5.4 $\pm$ 1.8 *	1.9 $\pm$ 0.6 #	2.5 $\pm$ 0.8 *#
<b>Glucagon</b> (pg/ml•120 min)	-18.9 $\pm$ 296	-731 $\pm$ 260 *	860 $\pm$ 306 #	413 $\pm$ 303 #
<b>GLP-1</b> (pM•240 min)	235 $\pm$ 50	520 $\pm$ 61 *	652 $\pm$ 83 #	1616 $\pm$ 239 *#
<b>GE lag period</b> (min)	80 $\pm$ 2.5	91 $\pm$ 3.8 *	69 $\pm$ 4.5 #	70 $\pm$ 3.8 #
<b>GE Half time</b> (min)	158 $\pm$ 6.5	198 $\pm$ 18 *	143 $\pm$ 6.0	152 $\pm$ 8.0 #

PP incremental AUC, Mean $\pm$ SEM, 2 way RM ANOVA. \* $P<0.05$  vs Placebo po, same iv infusion; # $P<0.05$  vs saline iv, same oral treatment. GE gastric emptying

Clinical Trial Registration Number: NCT00551590

Reported by: MSD

## 737

### Comparison of GLP-1R expression in C-cells isolated from rat and human thyroid glands

G. Dietert<sup>1</sup>, M. Dorau<sup>1</sup>, M. Heinrichs<sup>1</sup>, A. Perren<sup>2</sup>, A. Blank<sup>2</sup>, T. Kissner<sup>1</sup>;

<sup>1</sup>Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany,

<sup>2</sup>University of Bern, Switzerland.

**Background and aims:** In rodent thyroid glands, some glucagon-like peptide-1 receptor (GLP-1R) agonists have been shown to induce C-cell proliferation. Rodents appear to be particularly sensitive, and it remains to be determined whether this is a drug class effect. To evaluate if GLP-1R expression on C-cells is rodent-specific, we investigated GLP-1R expression levels in C-cells isolated from untreated human and rat thyroids.

**Materials and methods:** Rat and human C-cells were excised by laser capture microdissection from paraffin-embedded thyroid glands, and total RNA was isolated. Quantitative real-time polymerase chain reaction was performed to analyse GLP-1R gene expression levels; GLP-1R expression levels were normalized to  $\beta$ -actin levels.

**Results:** GLP-1R expression in rat C-cell fractions ( $n=15$ ) was reflected in a cycle time (CT) mean value of  $27.4 \pm 1.5$  and a value of 4 when normalized to  $\beta$ -actin (Table). In a study with human C-cell fraction ( $n=2$ ), only marginal GLP-1R expression levels were observed, reflected by a CT mean value of  $33.6 \pm 0.6$ , close to the sensitivity limit of the test system ( $CT=35$ ) and 10.4 when normalized to  $\beta$ -actin. In a second study with human tissue ( $n=7$ ), no relevant GLP-1R expression was demonstrated in C-cell fractions.

**Conclusion:** GLP-1R expression analysis in human and rat thyroid glands revealed higher GLP-1R expression in rat C-cells compared with human C-cells, with a CT difference of at least six CTs, corresponding to a 64-fold higher RNA content in rats. The higher GLP-1R expression in rats may underlie the particular sensitivity of rodents for GLP-1R agonist-induced proliferation of thyroid C-cells.

Expression of the GLP-1R in rat and human thyroid C-cell fractions

Target	Expression level, mean CT value* $\pm$ SD (range)		
	Study 1: Rat C-cells (n=15-18)	Study 2: Human C-cells (n=2)	Study 1: Human C-cells (n=7)
$\beta$ -actin <sup>†</sup>	23.5 $\pm$ 1.1 (21.7-24.9)	23.2 $\pm$ 0.4 (23.0-23.5)	24.6 $\pm$ 3.8 (19.3-30.6)
Calcitonin <sup>‡</sup>	18.1 $\pm$ 1.3 (16.4-21.8)	20.7 $\pm$ 1.0 (20.0-21.4)	26.6 $\pm$ 2.8 (22.8-30.4)
GLP-1R	27.4 $\pm$ 1.5 (24.1-29.5)	33.6 $\pm$ 0.6 (33.2-34.0)	Undetectable (all values >35, except one [34.9])
$\Delta$ CT GLP-1R <sup>§</sup>	4.0 $\pm$ 1.35	10.4 $\pm$ 0.23	Not applicable

\*Higher CT value=lower gene expression; <sup>†</sup>Expression of the housekeeping gene  $\beta$ -actin was used for quality control; <sup>‡</sup>Calcitonin gene expression was used as a marker for the presence of C-cells; <sup>§</sup>GLP-1R value was normalized to  $\beta$ -actin; GLP-1R=glucagon-like peptide-1 receptor; CT=cycle time; SD=standard deviation

Supported by: Sanofi

## 738

### Is introduction of GLP-1 agonist and hypocaloric diet justified and safe for obese type 2 diabetes patients treated with insulin?

M. Vrtovec;

Department of Endocrinology, University Medical Centre, Ljubljana, Slovenia.

**Background and aims:** Patients with type 2 diabetes and BMI over 35 kg/m<sup>2</sup> are at much higher risk than non obese patients. We sought to investigate whether the introduction of GLP-1 agonist together with hypocaloric diet (5 MJ) was safe for insulin treated patients with type 2 diabetes and BMI over 35 kg/m<sup>2</sup> and whether it resulted in lower HbA<sub>1c</sub> and greater weight reduction.

**Materials and methods:** 20 patients (9 women, 11 men) with type 2 diabetes and body mass index (BMI) over 35 kg/m<sup>2</sup> (42.8  $\pm$  7.3), treated with metformin and insulin, were observed. They were 41-78 (60.2  $\pm$  8.3) years old, with diabetes duration of 6-37 (16.4  $\pm$  8.8) years, treated with metformin and insulin for 1-11 years (4.7  $\pm$  2.6 years). At baseline 5 MJ diet was suggested, exenatide 5 mcg BID was introduced, short acting or 50% premixed insulins were switched to NPH or long acting analogs in reduced daily insulin dose (TDI) by 50%, 25%/30% mixtures of biphasic insulins twice daily were only reduced. The patients had to do a 7 point daily glucose profile before each visit and also between the 3rd and 7th day after the introduction of exenatide, in other days only FPG. After 1 month, exenatide dose was increased to 10 mcg BID. TDI was further reduced by 50% and the patients were instructed to adjust daily insulin dose at FBG levels below 8 mmol/l and over 10 mmol/L.

**Results:** After 6 months of treatment, we observed a significant decrease in HbA<sub>1c</sub> (from 9.4  $\pm$  1.3 % to 8.1  $\pm$  1.3 %,  $p < 0.05$ ), and an average 13 kg body weight reduction (123.5  $\pm$  22.4 kg to 110.7  $\pm$  18.0 kg,  $p < 0.05$ ). The average FPG was 10.3 mmol/L and the average of blood glucose profile 10.6 mmol/L. Insulin was withdrawn in 16 patients (80%), but to 9 of them sulphonylureas were prescribed later. Only 7 patients (35%) reported mild adverse events during the introduction of exenatide, and only one patient reported about 5 hypoglycemia in the first month, when he tried to reach FPG below 6 mmol/L.

**Conclusion:** Introduction of GLP-1 agonist together with hypocaloric diet in obese patients with diabetes type 2 is justified and safe. The expected benefits are better glycemic regulation, greater weight loss, much lower insulin dose and in some cases also withdrawal of insulin. Hypoglycaemia can occur in patients treated with insulin together with exenatide and hypocaloric diet if the target value of FBG is set to low.

## PS 056 SGLT-2 I

### 739

#### Ipragliflozin reduced HbA<sub>1c</sub> and body weight in Japanese type 2 diabetes patients who have inadequate glycaemic control on sulfonylurea or pioglitazone alone

A. Kashiwagi<sup>1</sup>, T. Shiga<sup>2</sup>, N. Akiyama<sup>2</sup>, K. Kazuta<sup>2</sup>, H. Ogasawara<sup>2</sup>, S. Yoshida<sup>2</sup>, E. Ueyama<sup>2</sup>, A. Utsuno<sup>2</sup>;

<sup>1</sup>Shiga University of Medical Science, <sup>2</sup>Astellas Pharma Inc., Tokyo, Japan.

**Background and aims:** Ipragliflozin (IPRA, ASP1941), a sodium glucose cotransporter 2 (SGLT2) inhibitor, successfully completed a phase 3 clinical monotherapy trial (EASD 2011; 149-OP) for the treatment of type 2 diabetes mellitus (T2DM) in Japanese patients. Here we report the results of 2 studies to assess the efficacy, safety, and tolerability of IPRA added to a sulfonylurea (SU) or pioglitazone (PIO) in Japanese T2DM patients who have inadequate glycaemic control while on a SU or PIO alone.

**Materials and methods:** In 2 independent, randomized, placebo-controlled, double-blind parallel group studies, patients entered a 4-week screening period followed by a 2-week single blind run-in period, and were randomized to either IPRA or placebo at a 2:1 ratio. Patients received 50 mg IPRA or placebo once daily in combination with either a SU (N = 242) or PIO (N = 151) at the constant approved dose of either any of the SUs or PIO in Japan.

**Results:** At week 24, IPRA significantly reduced HbA<sub>1c</sub> compared with placebo with a change of -1.14% in the SU and -0.88% in the PIO study, respectively ( $P < 0.001$ ; Table). In addition, more patients on IPRA reached target HbA<sub>1c</sub> of <7.0% (19.4% and 12.4%) compared with placebo (5.3% and 0.0%) in the SU and PIO studies respectively. Fasting plasma glucose (FPG) levels compared with placebo were also significantly decreased for both studies ( $P < 0.001$ ; Table). A significant decrease in body weight compared with placebo was observed in the IPRA group with a change of -1.32 kg for the SU study and -2.79 kg for the PIO study ( $P < 0.001$ ; Table). Also observed were decreases in systolic and diastolic blood pressure in the IPRA group compared with the placebo group. The incidences of hypoglycaemia, genital infections, and urinary tract infections with IPRA occurred in 1.2%, 0.6%, and 1.2% of patients in the SU study, respectively, and 1.0%, 2.1%, and 3.1% in the PIO study, respectively; the incidences in the placebo arm were respectively 1.3%, 3.9%, and 3.9% in the SU study and 0.0%, 0.0%, and 1.9% in the PIO study, respectively.

**Conclusion:** Ipragliflozin as a 50 mg once daily oral dose added to a SU or PIO during a 24-week period was efficacious and well tolerated in Japanese T2DM patients. Additional benefits of body weight and blood pressure reductions were also observed.

Table: Baseline and adjusted mean change from baseline at 24 weeks

		Sulfonylurea (N = 240)		Pioglitazone (N = 151)	
		Placebo (n = 75)	Ipragliflozin (n = 165)	Placebo (n = 54)	Ipragliflozin (n = 97)
HbA <sub>1c</sub> (%)	Baseline values mean ± SD	8.34 ± 0.727	8.38 ± 0.641	8.39 ± 0.644	8.24 ± 0.670
	Change from baseline mean ± SD	0.32 ± 0.963	−0.83 ± 0.717	0.22 ± 0.811	−0.64 ± 0.609
	Adjusted mean difference to placebo (95% CI) <sup>†</sup>	−1.14 (−1.348 to −0.936) <sup>‡</sup>		−0.88 (−1.108 to −0.648) <sup>‡</sup>	
FPG (mg/dL)	Baseline values mean ± SD	176.0 ± 35.54	179.7 ± 32.27	170.0 ± 29.18	172.9 ± 36.80
	Change from baseline mean ± SD	−1.0 ± 40.20	−41.4 ± 30.80	6.1 ± 30.99	−36.4 ± 33.35
	Adjusted mean difference to placebo (95% CI) <sup>†</sup>	−38.0 (−45.27 to −30.75) <sup>‡</sup>		−41.0 (−50.34 to −31.66) <sup>‡</sup>	
Body weight (kg)	Baseline values mean ± SD	63.90 ± 11.39	68.77 ± 12.39	72.99 ± 15.69	73.17 ± 13.41
	Change from baseline mean ± SD	−0.88 ± 1.79	−2.33 ± 2.15	0.51 ± 2.19	−2.29 ± 2.05
	Adjusted mean difference to placebo (95% CI) <sup>†</sup>	−1.32 (−1.884 to −0.754) <sup>‡</sup>		−2.79 (−3.499 to −2.091) <sup>‡</sup>	

\*The value for HbA<sub>1c</sub> (%) is estimated as an NGSP value (%) calculated by the formula: HbA<sub>1c</sub> (%) = 1.02 × HbA<sub>1c</sub> (JDS [%]) + 0.25%, considering the relational expression of HbA<sub>1c</sub> (JDS [%]) measured by the previous Japanese standard substance and measurement methods and HbA<sub>1c</sub> (NGSP).

NGSP, the National Glycohemoglobin Standardization Program; JDS, the Japan Diabetes Society

<sup>†</sup>Based on an analysis of covariance model that includes baseline HbA<sub>1c</sub>, FPG, or body weight as a covariate and treatment group as a fixed effect.

<sup>‡</sup>P < 0.001.

CI, confidence interval; FPG, fasting plasma glucose; SD, standard deviation.

Clinical Trial Registration Number: NCT01242215 and NCT01225081

## 740

### Luseogliflozin (TS-071), a selective SGLT2 inhibitor, improves glycaemic control and lowers body weight in a 12-week study in Japanese patients with type 2 diabetes mellitus

Y. Seino<sup>1</sup>, T. Sasaki<sup>2</sup>, A. Fukatsu<sup>3</sup>, Y. Samukawa<sup>4</sup>, S. Sakai<sup>4</sup>, T. Watanabe<sup>4</sup>;

<sup>1</sup>Kansai Electric Power Hospital, Osaka, <sup>2</sup>Jikei University School of Medicine, Tokyo, <sup>3</sup>Yachiyo Hospital, Aichi, <sup>4</sup>Taisho Pharmaceutical Co., Ltd., Tokyo, Japan.

**Background and aims:** Renal sodium-glucose co-transporter (SGLT) 2 inhibition is a new approach to the treatment of type 2 diabetes mellitus (T2DM). Luseogliflozin (LUSEO) is orally bioavailable and is a highly selective SGLT2 inhibitor with an IC<sub>50</sub> value of 2.26 nmol/L, and exhibits >1000-fold selectivity over SGLT1. In a previous exploratory 12 weeks study, once daily administration of LUSEO demonstrated clinical amelioration in HbA<sub>1c</sub> and other glycaemic parameters, and showed a favourable safety profile. The present study was designed to investigate the efficacy and safety of LUSEO monotherapy in Japanese patients with T2DM.

**Materials and methods:** In this double-blind, placebo (PBO)-controlled, parallel group, dose-finding study, 280 patients were randomised to LUSEO 1, 2.5, 5, or 10 mg once daily or PBO for 12 weeks. Patients aged 20–74 years with HbA<sub>1c</sub> 6.9–10.4 %, who had received regular diet therapy but no antidiabetic drugs for the twelve weeks before randomisation, were recruited to this study. The primary endpoint was change in HbA<sub>1c</sub> from baseline at end of treatment. The secondary endpoints were changes in fasting plasma glucose (FPG), postprandial plasma glucose (PPG) and body weight from baseline at end of treatment. Safety and tolerability were also evaluated.

**Results:** Baseline demographics and disease characteristics were similar among all groups. Mean baseline characteristics in each group were HbA<sub>1c</sub> 7.77–8.05%, FPG 149.3–158.9 mg/dL, PPG at 2-hours after meal test 245.2–257.7 mg/dL, and body weight 61.0–72.6 kg. After 12 weeks administration of LUSEO 1 mg–10 mg, HbA<sub>1c</sub>, FPG, 2-hour PPG and body weight were decreased significantly versus PBO (Table 1). There was a significant decrease in systolic blood pressure reductions without a relevant change in pulse rate in all LUSEO groups. The frequency of adverse events was similar in all groups. No major or serious safety concern was observed in any of the LU-

SEO groups. No hypoglycaemia (less than 70 mg/dL blood glucose level) was observed. Although 18 pollakisuria or urine output increases were observed in all LUSEO groups, all of these events were mild in severity.

Table 1. LS mean change from baseline at the end of trial.

	PBO (n=56)	LUSEO 1 mg (n=55)	LUSEO 2.5 mg (n=55)	LUSEO 5 mg (n=54)	LUSEO 10 mg (n=58)
HbA <sub>1c</sub> (%)	0.23	−0.29	−0.42	−0.46	−0.43
difference from PBO	−	−0.52 *	−0.65 *	−0.69 *	−0.66 *
FPG (mg/dL)	8.9	−10.6	−18.6	−21.1	−21.1
difference from PBO	−	−19.6 *	−27.6 *	−30.1 *	−30.1 *
2-hour PPG (mg/dL)	5.1	−44.7	−54.9	−55.5	−43.6
difference from PBO	−	−49.8 *	−60.0 *	−60.5 *	−48.6 *
Body weight (kg)	0.15	−0.81	−1.39	−1.97	−1.90
difference from PBO	−	−0.96 *	−1.54 *	−2.12 *	−2.05 *

ANCOVA with baseline as covariate for HbA<sub>1c</sub>, FPG and 2-hour PPG, ANOVA for Body weight. \*: P<0.001 vs. PBO

**Conclusion:** LUSEO ameliorated the glycaemic parameters and induced bodyweight reduction. Glycaemic control by LUSEO at 2.5 mg or higher doses was similar, and LUSEO showed a favourable safety profile, as was found in the previous exploratory study.

## 741

### Luseogliflozin (TS-071), a selective SGLT2 inhibitor, improves glycaemic control in Japanese type 2 diabetic subjects with renal impairment

M. Haneda<sup>1</sup>, Y. Seino<sup>2</sup>, A. Fukatsu<sup>3</sup>, T. Sasaki<sup>4</sup>, Y. Samukawa<sup>5</sup>, S. Sakai<sup>5</sup>, Y. Sato<sup>5</sup>, T. Watanabe<sup>5</sup>;

<sup>1</sup>Asahikawa Medical University, Hokkaido, Japan, <sup>2</sup>Kansai Electric Power Hospital, Osaka, Japan, <sup>3</sup>Yachiyo Hospital, Aichi, <sup>4</sup>Jikei University School of Medicine, Tokyo, <sup>5</sup>Taisho Pharmaceutical Co., Ltd., Tokyo, Japan.

**Background and aims:** SGLT2 inhibition is known as a new approach for the treatment of type 2 diabetes mellitus (T2DM). Luseogliflozin (LUSEO) is a highly selective inhibitor of SGLT2. This study was conducted to evaluate the effect of LUSEO on urinary glucose excretion (UGE) and plasma glucose levels, the pharmacokinetics, and safety in Japanese T2DM subjects with renal impairment and normal renal function.

**Materials and methods:** Japanese T2DM subjects with normal renal function (NRF [eGFR (mL/min/1.73m<sup>2</sup>)] 90–), and mild (G2 [eGFR 60–89]), moderate (G3 [eGFR 30–59]), and severe (G4 [eGFR 15–29]) renal impairment received a single oral dose of LUSEO (5 mg) before breakfast (n = 57). Pharmacodynamic parameters were measured to compare the values before and after administration of LUSEO, and pharmacokinetic parameters were compared between the administration groups. The per cent inhibition of glucose reabsorption by the kidney in all groups was analysed.

**Results:** Mean 24-hour urinary glucose excretion was significantly increased from baseline levels in all groups (ΔUGE (g, mean ± SD): NRF, 88.3 ± 36.9; G2, 69.7 ± 19.1; G3, 45.3 ± 16.8; G4, 21.8 ± 7.1). The values of postprandial plasma glucose (PPG: 2 hours after breakfast) were significantly decreased from baseline levels in all groups, except G4 (ΔPPG (mg/dL): NRF, −49.3 ± 33.6; G2, −33.6 ± 27.1; G3, −24.4 ± 34.7; G4, −3.3 ± 32.1). Moreover, the values of fasting plasma glucose (FPG) 24 hours after administration of LUSEO were also significantly decreased from baseline levels in all groups, except G4 (ΔFPG (mg/dL): NRF, −28.7 ± 22.0; G2, −16.2 ± 18.3; G3, −7.7 ± 13.4; G4: 10.3 ± 12.1). The average inhibition of glucose reabsorption by the kidney after LUSEO administration was almost 36% in all groups. LUSEO-induced increases in UGE were dependent on filtered glucose load, which is directly proportional to eGFR. Systemic exposure (AUC<sub>inf</sub> of plasma LUSEO concentration) was comparable among all groups. No serious adverse event or renal/urinary tract adverse event was observed during the study period.

**Conclusion:** LUSEO may be effective and may not require dose adjustment in the treatment of T2DM subjects even with mild to moderate renal impairment. A long-term clinical trial to assess the efficacy of LUSEO in T2DM subjects with moderate renal impairment is in progress.



## 742

# Luseogliflozin (TS-071), a novel, potent, and selective inhibitor of SGLT2 is safe and well-tolerated in type 2 diabetes mellitus and showed dose-dependent PK/PD in both US/Japanese ethnicities

L.A. Morrow<sup>1</sup>, M.J. Gutierrez<sup>2</sup>, Y. Tanaka<sup>3</sup>, H.D. Sabia<sup>3</sup>, K. Shimasaki<sup>3</sup>, H. Ochiai<sup>4</sup>, S. Yasuda<sup>4</sup>, Y. Samukawa<sup>4</sup>, M. Hompesch<sup>1</sup>, D.P. Rossignol<sup>2</sup>;

<sup>1</sup>Profil Institute for Clinical Research, Chula Vista, USA, <sup>2</sup>Comprehensive Clinical Development, Miramar, USA, <sup>3</sup>Taisho Pharmaceutical R&D USA, Morristown, USA, <sup>4</sup>Taisho Pharmaceutical CO., LTD., Tokyo, Japan.

**Background and aims:** Luseogliflozin (LUSEO) has previously been shown to enhance urine glucose excretion (UGE) and lower plasma glucose in studies with Japanese (JP) subjects with type 2 diabetes mellitus (T2DM). Currently we demonstrate that LUSEO is safe, tolerable, and active in US subjects with T2DM.

**Materials and methods:** This double blind, randomized, multiple ascending dose, placebo (PBO) controlled study assessed the pharmacokinetics (PK), pharmacodynamics (PD), safety and tolerability of LUSEO after q.d. oral dosing for 7 days in 72 subjects with T2DM in the US (HbA1c: 8.2±0.85%, age: 55±8.3, body weight (BW): 84±17 kg, gender: 50% female, ethnicity: 87.5% white). Study cohorts received 1, 2.5, 5, 10, 15 or 25 mg LUSEO or PBO prior to breakfast, 10:2 in each cohort.

**Results:** PK parameters (C<sub>max</sub> and AUC) of LUSEO were dose proportional and reached steady state by Day 3. All doses stimulated UGE throughout the dosing period, and was strongly dose-dependent to 2.5 mg/day (Figure 1). Modeling of UGE predicted a 50% effective dose <1 mg, and E<sub>max</sub> (101 g/day) corresponded to 10 mg LUSEO (109 ± 36.4 g/day). Comparing Day 7 to baseline, 10 mg LUSEO decreased plasma glucose exposure by 25% (P=0.0004 vs PBO) and mean plasma glucose (MPG) by 60 mg/dL (28%: P=0.0003 vs PBO). There were no serious treatment-emergent adverse events (TEAE); no AE leading to discontinuation; no dose dependent TEAE, including the most common TEAE (GI: 21%: 1 moderate) and no clinically-defined hypoglycemia. Urinary AEs were rare (1 incidence each of pollakiuria and polyuria, no urinary tract infections). LUSEO dosing was associated with enhanced weight loss (1.3 -2 vs 0.8 kg in PBO). In comparison to a prior study with JP subjects, C<sub>max</sub> and AUC were decreased in US subjects. Further analysis indicated that these differences may be related to differences in BW which were different in the two study populations (JP BW: 71±12 kg). In both US and JP populations, mean UGE was roughly similar up to doses of 5 mg. While effects on plasma glucose were affected by baseline disease severity in these small short-duration studies, preliminary findings of reductions in MPG and fasting plasma glucose appear to be optimal at 2.5 mg/day in JP subjects, but may require higher doses in US subjects.

**Conclusion:** Repeated dosing of LUSEO for 7 days demonstrated dose proportional PK at doses up to 25 mg, predictable PD, and was safe and well-tolerated in subjects with T2DM in the US. As a function of dose, exposure was lower in US subjects than in JP subjects, suggesting that treatment of US patients is likely to require higher doses of LUSEO than JP patients. Ultimately, establishing optimal dosing will require the use of more robust biomarkers of glucose exposure such as HbA1c.

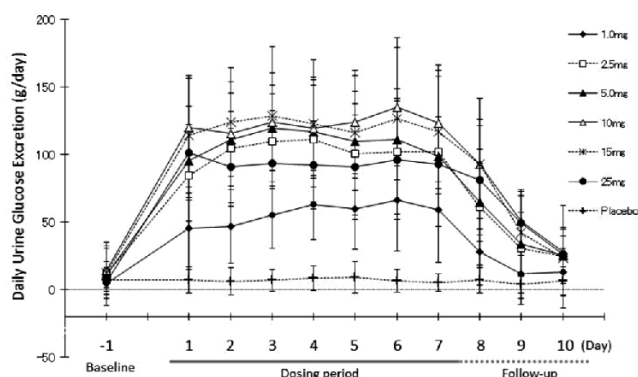


Figure 1: Daily Urine Glucose Excretion

Supported by: Taisho Pharmaceutical USA

## 743

# Safety of dapagliflozin in clinical trials for type 2 diabetes mellitus

K. Johnson<sup>1</sup>, A. Ptaszynska<sup>2</sup>, A. Apanovitch<sup>3</sup>, J.E. Sugg<sup>4</sup>, S.J. Parikh<sup>4</sup>, J.F. List<sup>2</sup>;

<sup>1</sup>AstraZeneca, Mölndal, Sweden, <sup>2</sup>Bristol-Myers Squibb, Princeton, USA,

<sup>3</sup>Bristol-Myers Squibb, Pennington, USA, <sup>4</sup>AstraZeneca, Wilmington, USA.

**Background and aims:** Dapagliflozin (DAPA), a sodium glucose cotransporter 2 (SGLT2) inhibitor, reduces hyperglycaemia in an insulin-independent manner by increasing renal glucose excretion, and represents a potential option for T2DM patients. The aim of this analysis was to assess safety across the DAPA clinical development programme.

**Materials and methods:** Data were pooled from 12 phase 2b/3 short-term double-blind placebo (PBO)-controlled trials (4684 patients) to examine overall safety. An updated database of 19 trials (8685 patients; 1.8:1 cumulative exposure ratio for DAPA:control) was used to examine rare events.

**Results:** Adverse events (AEs) were slightly more common with DAPA vs PBO; serious AEs and discontinuations due to AEs were balanced across groups (Table 1). Hypoglycaemia was more common with DAPA vs PBO, driven by studies in which DAPA was combined with sulphonylurea or insulin. AEs of genital infections and UTI were more common with DAPA vs PBO with imbalances less marked for UTIs. These were mild/moderate, responded to standard antimicrobial therapy and rarely led to discontinuation. Mean changes from baseline in systolic/diastolic BP were -4.0/-2.0 vs -0.9/-0.5 mmHg for DAPA vs PBO without increases in measured orthostatic hypotension (3.9% vs 3.7% for DAPA vs PBO). AEs of volume depletion (hypotension/dehydration/hypovolaemia) were seen in 0.6-1.2% for DAPA 0.4% for PBO. AEs of renal impairment/failure were balanced across groups (0.9-1.4% for DAPA vs 0.9% for PBO). Minor changes in serum electrolytes were seen with DAPA, with small increases in serum Mg and P. PBO-corrected reductions in uric acid, in the studies it was analysed, ranged from -0.45 to -0.8 mg/dL (but reductions of less magnitude were noted in an add-on to insulin and a phase 2b monotherapy study). Incidence rates/100 pt years of malignant and unspecified tumours were similar for DAPA (1.39) vs control (1.34). Incidence rates of bladder cancer were 0.15 for DAPA vs 0.03 for control with an incidence rate (IR) difference of 0.117 (95%CI: -0.171, 0.367) and of breast cancer were 0.37 vs 0.22 respectively with an IR difference of 0.228 (95%CI: -0.537%, 0.806). Haematuria was noted at or before baseline in 7/10 patients with bladder cancer. All breast cancer events were reported within the first year of study initiation. Non-clinical DAPA data did not indicate any carcinogenic potential, and SGLT2 is selectively expressed in the kidney with no expression in human breast or bladder tissue. Elevated liver laboratory tests were seen in 4.4% vs 4.2% for DAPA vs control. Hazard ratio for CV death, MI, stroke or hospitalization for unstable angina for DAPA vs control was 0.82 (95% CI: 0.583, 1.152). Trends in CV events and specific malignancies will be further evaluated in a randomised CV outcomes trial and complementary observational studies.

**Conclusion:** Based on this large phase 2b/3 experience with >4500 T2DM patients, DAPA has an acceptable safety profile.

Table 1. Safety summary, short-term double-blind PBO-controlled pool

	Number (%) of patients			
	Placebo (N=1393)	DAPA 2.5 mg (N=814)	DAPA 5 mg (N=1145)	DAPA 10 mg (N=1193)
≥ 1 AE	792 (56.9)	493 (60.6)	709 (61.9)	734 (61.5)
≥ 1 SAE	46 (3.3)	37 (4.5)	40 (3.5)	42 (3.5)
Deaths	1 (0.1)	1 (0.1)	2 (0.2)	3 (0.3)
SAE leading to disc of study med.	11 (0.8)	5 (0.6)	9 (0.8)	9 (0.8)
AE leading to disc of study med.	35 (2.5)	18 (2.2)	32 (2.8)	38 (3.2)
Hypoglycaemia leading to disc of study med.	0	0	0	0
Events of special interest				
≥ 1 hypoglycaemia	112 (8.0)	133 (16.3)	130 (11.4)	128 (10.7)
Events of UTI	3.7%	3.6%	5.7%	4.3%
Events of genital infections	0.9%	4.1%	5.7%	4.8%

Clinical Trial Registration Number: NCT00263276; NCT00736879; NCT00972244; NCT00528372; NCT00528879; NCT00855166; NCT00357370; NCT00680745; NCT01095666; NCT00673231; NCT00643851; NCT00859898; NCT00663260; NCT00831779; NCT01031680; NCT01042977

Supported by: AZ and BMS

## 744

**Dapagliflozin improves glycaemic control and reduces body weight across various patient populations with type 2 diabetes mellitus**

S. Parikh<sup>1</sup>, E. Hardy<sup>1</sup>, L. Wei<sup>2</sup>, C. Wessman<sup>3</sup>, A. Salsali<sup>4</sup>, A. Ptaszynska<sup>4</sup>,  
<sup>1</sup>AstraZeneca, Wilmington, USA, <sup>2</sup>Bristol-Myers Squibb, Pennington, USA,  
<sup>3</sup>AstraZeneca, Mölndal, Sweden, <sup>4</sup>Bristol-Myers Squibb, Princeton, USA.

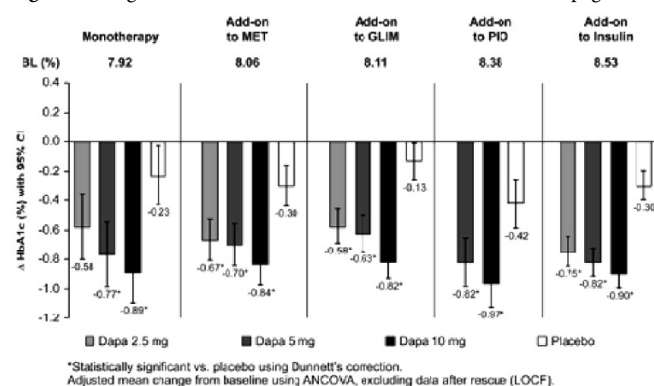
**Background and aims:** Dapagliflozin (DAPA), a selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, reduces plasma glucose independently of insulin by increasing renal glucose excretion. The efficacy of DAPA in patients with T2DM across 5 clinical trials was assessed.

**Materials and methods:** Patients with T2DM received DAPA (2.5, 5, or 10 mg/d) or placebo for 24 wk as monotherapy (N=274, main cohort) or add-on to metformin (MET, N=546), glimepiride (GLIM, N=596), pioglitazone (PIO, N=420) or insulin (INS, N=807). The primary endpoint for all trials was mean change from baseline in HbA1c at week 24. Secondary endpoints included change from baseline in fasting plasma glucose (FPG), postprandial glucose (PPG, tested in add-on to GLIM and add-on to PIO studies), and body weight. The primary endpoint was analyzed by ANCOVA, excluding data after rescue (last observation carried forward). Secondary endpoints were analyzed by hierarchical testing.

**Results:** Mean baseline HbA1c ranged from 7.9% to 8.5%. Placebo-corrected adjusted mean changes from baseline in HbA1c for DAPA 10 mg/d ranged from -0.5% to -0.7% ( $P<0.001$  in all studies) (Figure). DAPA also reduced FPG in all studies, and PPG in the 2 trials where it was tested. Body weight was reduced by 1.5 to 2 kg with DAPA 10 mg/d compared with placebo in the add-on to MET, add-on to GLIM, add-on to PIO, and add-on to insulin trials ( $P<0.0001$  in all 4 studies). Placebo-corrected weight reductions of up to 1 kg were observed in the monotherapy trial, however these changes did not reach statistical significance due to a greater-than-expected weight effect in the placebo group. Dapagliflozin was generally safe and well tolerated in these trials. Signs and symptoms suggestive of urinary tract infection and genital infection were more commonly reported as adverse events in the dapagliflozin dose groups compared with placebo.

**Conclusion:** DAPA produced significant improvements in glycaemic control and body weight in various patient populations with T2DM across the treatment continuum, consistent with its insulin-independent mechanism of action.

**Figure.** Change from baseline in HbA1c in Phase 3 studies of dapagliflozin



Clinical Trial Registration Number: NCT00528372; NCT00528879;  
 NCT00680745; NCT00683878; NCT00673231  
 Supported by: AZ and BMS

## 745

**Efficacy and safety of dapagliflozin treatment for type 2 diabetes mellitus patients with comorbid cardiovascular disease and hypertension**

W. Cefalu<sup>1</sup>, L. Leiter<sup>2</sup>, T. DeBruin<sup>3</sup>, I. Gause-Nilsson<sup>4</sup>, J. Sugg<sup>3</sup>, S. Parikh<sup>3</sup>,  
<sup>1</sup>University of Louisiana, Baton Rouge, USA, <sup>2</sup>University of Toronto, Canada,  
<sup>3</sup>AstraZeneca, Wilmington, USA, <sup>4</sup>AstraZeneca, Mölndal, Sweden.

**Background and aims:** Dapagliflozin (DAPA), a selective SGLT2 inhibitor, is a glucuretic agent that reduces hyperglycaemia independent of insulin (INS) secretion or action. The mechanism of action and clinical profile of DAPA suggest that it may benefit patients with advanced type 2 diabetes mellitus (T2DM) and cardiovascular (CV) comorbidities. This study was 1 of 2 assess-

ing DAPA in this population. Enrolled patients had HbA1c  $\geq 7.0\%$ – $\leq 10.0\%$ , documented CV disease (CVD), and a history of hypertension (HTN).

**Materials and methods:** Randomised patients (N=922), aged  $\geq 45/50$  y (M/F), received double-blind DAPA 10 mg/d or placebo (PBO) for 24 wks added to usual care for T2DM and HTN (the 80-wk extension is ongoing). Patients receiving INS had their baseline (BL) INS dose reduced by 25%. Rescue medication was provided as required for glycaemic or HTN control. Patients were stratified by age, INS use, and time from the most recent qualifying CV event. Co-primary end points were change in HbA1c and patients (%) achieving a composite end point consisting of a reduction of HbA1c  $\geq 0.5\%$ , body weight (BW)  $\geq 3\%$ , and systolic blood pressure (SBP)  $\geq 3$  mm Hg.

**Results:** The mean age was 63 y; 42% were  $\geq 65$  y. Mean T2DM duration was 12 y. At BL, 41% of patients received 2 oral anti-hyperglycaemic agents. INS was used by 52% of patients, of which 17% used INS as the only treatment. The most common qualifying CV event was coronary heart disease (75%). Almost 13% of the patients had chronic heart failure. The duration of HTN was  $\geq 3$  y in 88% of the patients and  $\geq 10$  y in 50% of the patients. Greater reductions from BL occurred with DAPA vs PBO in HbA1c (BL 8.1%) and BW (BL 93.1 kg) and more patients in the DAPA group met the 3-item composite end point (Table). SBP (BL 133.2 mmHg) was reduced for DAPA vs PBO at 8 and 24 wks. The mean daily INS dose change for DAPA was 1.0 IU/d vs PBO 5.1 IU/d, nominal  $P<0.05$ . Age-stratified results ( $<65$  or  $\geq 65$  y) were similar to overall results, except SBP reduction, which did not differ significantly between DAPA and PBO groups for patients aged  $\geq 65$  y. The proportion of patients with serious adverse events (AEs, DAPA 5.9% vs PBO 5.6%), unadjudicated CV events reported as AEs (DAPA 5.2% vs PBO 4.8%) or serious AEs (DAPA 1.1% vs PBO 1.5%), and hypoglycaemia (DAPA 19.3% vs PBO 18.2%) were balanced between groups. More patients in the DAPA (17.2%) vs the PBO group (13.2%) had measured orthostatic HTN. Events of genital infections were more often seen in women than men, and more often reported for DAPA vs PBO. Events of urinary tract infections were similar for both groups.

**Conclusion:** DAPA, when added to standard of care in a 24-wk study of older T2DM patients with mean age  $>60$  y and comorbid CVD and HTN, while improving glycaemic and BP control, and reducing BW, did not adversely impact CV safety or frequency of hypoglycaemic events. These data indicate that DAPA is appropriate for use in patients with advanced T2DM and CVD.

	PBO N=459	DAPA 10 mg N=455
HbA1c, % <sup>a</sup>	0.08 (0.04)	-0.38 (0.04)*
3-item composite end point, % <sup>b</sup>	0.9%	11.7%*
SBP, 8 wks, mmHg <sup>a</sup>	-0.99 (0.67)	-2.96 (0.68)†
SBP, 24 wks, mmHg <sup>a</sup>	-1.03 (0.69)	-2.99 (0.70)†
BW, % change <sup>a</sup>	-0.30 (0.16)	-2.56 (0.16)*

<sup>a</sup>Data are adjusted mean change from BL (SE), last observation carried forward. <sup>b</sup>Data are adjusted proportion, last observation carried forward.  
 \* $P<0.0001$ ; † $P<0.02$ .

Clinical Trial Registration Number: NCT01031680  
 Supported by: AstraZeneca and Bristol-Myers Squibb

## 746

**Efficacy of dapagliflozin compared with other oral antidiabetic agents added to metformin monotherapy among subjects with type 2 diabetes**

S.M. Goring<sup>1</sup>, T. Huang<sup>1</sup>, G. Wygant<sup>2</sup>, M. Grishchenko<sup>3</sup>, R. Townsend<sup>4</sup>,  
 A. Salsali<sup>2</sup>, I. Wood<sup>2</sup>, N. Hawkins<sup>6</sup>,  
<sup>1</sup>Oxford Outcomes, Vancouver, Canada, <sup>2</sup>Bristol-Myers Squibb, Princeton, USA, <sup>3</sup>Bristol-Myers Squibb, Rueil-Malmaison, France, <sup>4</sup>AstraZeneca, Zaventem, Belgium, <sup>5</sup>Bristol-Myers Squibb, Uxbridge, UK, <sup>6</sup>Oxford Outcomes, UK.

**Background and aims:** Dapagliflozin is a novel sodium-glucose co-transporter 2 (SGLT2) inhibitor that has demonstrated utility as add-on therapy to metformin among patients with type 2 diabetes mellitus (T2DM). Its efficacy and safety have been compared in randomized clinical trials (RCTs) vs glipizide (a sulfonylurea [SU]) and placebo; however, head-to-head data vs other antidiabetic agents are not available. We used indirect evidence from a network of RCTs to estimate the efficacy of dapagliflozin relative to other antidiabetic agents after one year of exposure. Outcomes considered were mean change from baseline HbA1c and weight, at 52 weeks. As a measure of safety,

we estimated the relative odds of experiencing hypoglycemia during the first year of treatment.

**Materials and methods:** We conducted a systematic literature review and network meta-analysis (NMA) of RCTs involving antidiabetic agents added to metformin. We searched EMBASE, MEDLINE, and the Cochrane Central Register of Controlled Trials from database inception to May 2011 as well as abstracts from selected 2010 conferences, clinical trial registries, reference lists of included studies and unpublished study reports. We included RCTs that enrolled subjects with T2DM inadequately controlled on metformin monotherapy. RCTs involving intensive diet regimens were included in a sensitivity analysis. The analysis was performed using fixed- and random-effects Bayesian NMA models. Mean change from baseline HbA1c and weight after 52 weeks of treatment were analyzed on the mean difference scale. The proportion of subjects with at least one episode of hypoglycemia was analyzed on the odds ratio scale.

**Results:** We identified 4270 abstracts, and six RCTs met inclusion criteria. Four RCTs compared dipeptidyl peptidase-4 (DPP-4) inhibitors with SUs, one compared a thiazolidinedione (TZD) with a SU, and one compared dapagliflozin with a SU. Two RCTs were identified which compared glucagon-like peptide-1 (GLP-1) analogues with SUs, but neither was eligible for the primary analysis. After one year of treatment, the mean change from baseline in HbA1c was -0.69% in the SU arms. Anchored on this estimate, the estimated mean change was -0.69% (95% CrI: -0.85 to 0.53) for dapagliflozin, -0.67% (95% CrI: -0.84 to -0.51) for TZDs and -0.61% (95% CrI: -0.70 to -0.53) for DPP-4 inhibitors. Based on an average weight gain of 1.31 kg observed in the SU trial arms, the absolute weight loss estimate was -3.36 kg (95% CrI: -5.72 to -1.04) for dapagliflozin and -0.61 kg (95% CrI: -1.81 to 0.51) for DPP-4 inhibitors. The RCT involving TZDs did not report weight change. Odds ratios for hypoglycemia associated with dapagliflozin compared with TZDs and DPP-4 inhibitors were similar: 0.92 (95% CrI: 0.09 to 3.88) and 0.81 (95% CrI: 0.18 to 2.59), respectively. However, the odds for hypoglycemia associated with dapagliflozin were statistically lower than with SUs (OR: 0.06; 95% CrI: 0.02 to 0.17).

**Conclusion:** Compared with existing licensed antidiabetic therapies, dapagliflozin offers similar HbA1c control with either similar or reduced risk of hypoglycemia and the additional benefit of sustained weight loss over one year. Supported by: BMS

## 747

### Dapagliflozin reduces renal threshold for glucose excretion in type 2 diabetes

R.A. DeFronzo<sup>1</sup>, M. Hompesch<sup>2</sup>, S. Kasichayanula<sup>3</sup>, X. Liu<sup>3</sup>, Y. Hong<sup>3</sup>, M. Pfister<sup>3</sup>, L.A. Morrow<sup>2</sup>, B.R. Leslie<sup>3</sup>, D.W. Boulton<sup>4</sup>, A. Ching<sup>3</sup>, F.P. LaCreta<sup>3</sup>, S.C. Griffen<sup>3</sup>

<sup>1</sup>University of Texas Health Science Center, San Antonio, <sup>2</sup>Profil Institute for Clinical Research, Chula Vista, <sup>3</sup>Bristol-Myers Squibb, Princeton, <sup>4</sup>Bristol-Myer Squibb, Princeton, USA.

**Background and aims:** Dapagliflozin is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor that reduces hyperglycaemia in type 2 diabetes by promoting urinary glucose excretion. This study examined the effect of dapagliflozin on renal glucose kinetics in healthy subjects and subjects with type 2 diabetes.

**Materials and methods:** Renal glucose kinetics were assessed in 12 healthy control subjects (HbA1c 5.5 %, 41 years, 27 kg/m<sup>2</sup>, 7 male/5 female) and 12 subjects with type 2 diabetes (HbA1c 6.5%, 53 years, 30 kg/m<sup>2</sup>, 7 male/5 female). All subjects had an estimated glomerular filtration rate (eGFR) ≥ 60 and ≤ 160 mL/min-1.73 m<sup>2</sup> by the Modification of Diet in Renal Disease equation. Subjects underwent a combined pancreatic and stepped hyperglycaemic clamp at baseline and after 7 days of dapagliflozin 10 mg/day. The pancreatic/stepped hyperglycaemic clamp was performed with octreotide and basal insulin/glucagon/growth hormone replacement, and evaluated a plasma glucose range of 100–550 mg/dL. Subjects with type 2 diabetes continued on baseline therapy unless taking metformin, which was held for 48 h before the pancreatic stepped hyperglycaemic clamp. A model was developed to describe maximum tubular glucose transport (TmG), renal glucose threshold (for onset of glucosuria), and splay of the glucose titration curve for healthy and type 2 diabetes populations, and then used to estimate these parameters from individual titration curves.

**Results:** At baseline, the TmG (443 vs 326 mg/min,  $P < 0.04$ ) and splay (28,650 vs 14,248 mg<sup>2</sup>/min<sup>2</sup>,  $P < 0.0001$ ) were significantly higher in subjects with type 2 diabetes versus controls. The baseline threshold was similar in subjects with type 2 diabetes (196 mg/dL [10.9 mmol/L]) and controls (171 mg/dL [9.5

mmol/L]). Dapagliflozin treatment reduced TmG by 58% in type 2 diabetes subjects and 53% in healthy subjects. The splay declined similarly by ~37% for both groups. Following dapagliflozin, the modelled threshold was markedly reduced in type 2 diabetes patients, by 89% (21 mg/dL [1.2 mmol/L]) and in healthy controls, by 78% (37 mg/dL [2.1 mmol/L]). These modelled threshold values were well below the plasma glucose levels measured in this study. A modest reduction in GFR (~14%) was observed following dapagliflozin treatment in both groups.

**Conclusion:** In summary, type 2 diabetes subjects have a higher TmG and splay than healthy controls. Dapagliflozin treatment for 7 days reduced TmG and splay. However, the markedly decreased renal glucose threshold was the primary mechanism responsible for the induction of glucosuria with dapagliflozin in both subjects with type 2 diabetes and healthy controls.

Clinical Trial Registration Number: NCT01165268

Supported by: AstraZeneca and Bristol-Myers Squibb

## 748

### Dapagliflozin has no long-term effect on markers of bone turnover or bone mineral density in patients with inadequately controlled type 2 diabetes on metformin

Ö. Ljunggren<sup>1</sup>, J. Bolinder<sup>2</sup>, L. Johansson<sup>1,3</sup>, J.P.H. Wilding<sup>4</sup>, A.M. Langkilde<sup>3</sup>, C.D. Sjöström<sup>3</sup>, J. Sugg<sup>5</sup>, S. Parikh<sup>5</sup>

<sup>1</sup>Uppsala University, Sweden, <sup>2</sup>Karolinska Institute, Stockholm, Sweden,

<sup>3</sup>AstraZeneca, Mölndal, Sweden, <sup>4</sup>University of Liverpool, UK, <sup>5</sup>AstraZeneca, Wilmington, USA.

**Background and aims:** Dapagliflozin, a selective inhibitor of sodium-glucose cotransporter 2 (SGLT2), reduces hyperglycaemia in patients with type 2 diabetes mellitus (T2DM) by increasing urinary glucose excretion. Owing to its renal tubular mechanism of action, and because small increases in serum magnesium, phosphorus and parathormone without changes in serum calcium have been noted in clinical trials of dapagliflozin, we evaluated markers of bone formation and resorption and bone mineral density (BMD) in patients whose T2DM was inadequately controlled on metformin after 102 weeks of dapagliflozin treatment.

**Materials and methods:** This international, multi-centre, randomised, parallel-group, double-blind, placebo-controlled study enrolled patients with T2DM (mean values: women 63.3 and men 58.6 years; HbA1c 7.17%; BMI 31.9 kg/m<sup>2</sup>; and body weight 91.5 kg) inadequately controlled on metformin. A total of 182 patients were randomly assigned 1:1 to receive dapagliflozin 10 mg/day or placebo added to open-label metformin for a 24-week double-blind treatment period followed by a 78-week site- and patient-blinded extension period. At week 102 the following safety variables were evaluated: serum markers of bone formation (procollagen type 1 N-terminal propeptide; P1NP) and resorption (C-terminal crosslinking telopeptides of type I collagen; CTX); BMD at lumbar spine (L1-4), femoral neck and total hip regions assessed using standardised dual-energy x-ray absorptiometry (DXA); and adverse events of fracture.

**Results:** A total of 140 (76.9%) patients completed the 102-week study. Dapagliflozin vs placebo difference in adjusted mean change from baseline at 102 weeks for body weight was -2.42 kg (95% CI -3.64, -1.21) and for HbA1c was -0.42% (95% CI -0.62, -0.22). Baseline HbA1c was 7.16% and 7.19% in the dapagliflozin vs placebo groups, respectively. At 102 weeks, no statistically significant dapagliflozin vs placebo differences were identified in mean changes from baseline in P1NP (1.16 µg/L,  $p = 0.4096$ ), CTX (0.01 µg/L,  $p = 0.6916$ ) or mean percent changes from baseline in BMD at lumbar spine (0.22,  $p = 0.7013$ ), femoral neck (-0.94,  $p = 0.1521$ ) or total hip (-0.45,  $p = 0.3105$ ) regions. Over 102 weeks, no dapagliflozin vs placebo differences in proportions of patients with a BMD decrease of ≥5% were evident in any anatomical region (Table). There were no clinically significant changes in serum calcium, parathormone or 25-OH vitamin D; however, serum inorganic phosphorus increased. One patient in each treatment group experienced a forearm fracture.

**Conclusion:** Compared with placebo, dapagliflozin had no effect on markers of bone formation and resorption or BMD after 102 weeks of treatment in patients whose T2DM was inadequately controlled on metformin.



Changes in markers of bone turnover and BMD with dapagliflozin over 102 weeks

	Placebo + Metformin (N/n = 91/71)	Dapagliflozin 10mg + Metformin (N/n = 91/69)
<b>PINP, µg/L</b>		
Baseline mean (SD)	27.36 (13.06)	26.98 (10.44)
Change at 102w†	0.50 (−1.88,2.88)	1.66 (−0.90,4.22)
Difference vs placebo	.	1.16 (−2.16,4.48)*
<b>CTX, µg/L</b>		
Baseline mean (SD)	0.23 (0.13)	0.22 (0.12)
Change at 102w†	0.02 (−0.00,0.04)	0.02 (0.00,0.05)
Difference vs placebo	.	0.01 (−0.02,0.04)*
<b>BMD</b>		
<b>at lumbar spine (L1-4)</b>		
Baseline–mean(SD),g/cm <sup>2</sup>	1.19 (0.19)	1.18 (0.20)
% change at 102w‡	0.47 (−0.32,1.27)	0.69 (−0.19,1.57)
Difference vs placebo	.	0.22 (−0.89,1.34)*
<b>at femoral neck</b>		
Baseline–mean(SD),g/cm <sup>2</sup>	0.94 (0.14)	0.97 (0.14)
% change at 102w‡	0.09 (−0.83,1.01)	−0.85 (−1.85,0.16)
Difference vs placebo	.	−0.94 (−2.21,0.35)*
<b>at total hip</b>		
Baseline–mean(SD),g/cm <sup>2</sup>	1.05 (0.11)	1.10 (0.14)
% change at 102w‡	−0.37 (−1.00,0.26)	−0.82 (−1.52,−0.12)
Difference vs placebo	.	−0.45 (−1.32,0.43)*
<b>≥5% decrease in BMD</b>		
<b>at lumbar spine (L1-4)</b>		
Proportion over 102w§	5.9% (1.0, 10.9)	7.4% (1.7,13.1)
Difference vs placebo	.	1.4% (−6.1,9.0)
<b>at femoral neck</b>		
Proportion over 102w§	11.1% (4.3, 17.8)	11.3% (4.5,18.2)
Difference vs placebo	.	0.3% (−9.4,9.9)
<b>at total hip</b>		
Proportion over 102w§	4.7% (NA)	4.9% (NA)
Difference vs placebo	.	0.2% (−7.5,8.2)

N = the number of patients in the safety analysis set. n = the number of patients completing the study. \*p>0.15. Data are adjusted mean† and adjusted mean percent‡ changes from baseline and 95% CI derived from a mixed model. §Data are adjusted proportions and 95% CI derived from logistic regression (last observation carried forward), but with <5 events per treatment group, the exact method was used. BMD, bone mineral density; CTX, C-terminal crosslinking telopeptides of type I collagen. NA, not available; PINP, procollagen type I N-terminal propeptide.

Clinical Trial Registration Number: NCT00855166

Supported by: AZ and BMS

## PS 057 SGLT-2 II

749

### Influence of renal function on dapagliflozin pharmacokinetics and pharmacodynamics

S.C. Griffen, S. Kasichayanula, X. Liu, M. Pe Benito, M. Yao, F.P. LaCreta, M. Pfister, W.G. Humphreys, D.W. Boulton;  
Bristol-Myers Squibb, Princeton, USA.

**Background:** Dapagliflozin (DAPA), a selective inhibitor of the renal sodium glucose co-transporter 2 (SGLT2), improves hyperglycaemia by promoting urinary glucose excretion (UGE) proportional to the filtered load of glucose (plasma glucose × glomerular filtration rate [GFR]). The majority of DAPA is metabolised via glucuronidation by the enzyme UGT1A9 to the inactive metabolite, DAPA 3-O-glucuronide (D3OG), which is mainly cleared via renal excretion. As the kidney is the site of DAPA action, this open-label, parallel study was conducted to assess DAPA pharmacokinetics (PK), pharmacodynamics (PD), and safety in patients with type 2 diabetes (T2D) and renal impairment.

**Methods:** Patients with T2D and normal renal function or mild, moderate, or severe renal impairment (creatinine clearance of 51–80, 30–50, and < 30 mL/min excluding patients on dialysis, respectively) and healthy subjects received single (50 mg) and subsequent multiple (20 mg/day for 7 days - T2D patients only) doses of DAPA. Background antihyperglycaemic medications, excluding exenatide, were allowed.

**Results:** The PK profile of a single dose of DAPA 50 mg was similar in healthy subjects and patients with T2D and normal renal function. Exposure to DAPA and D3OG was incrementally higher with decreasing renal function (Table). After 7 days of DAPA 20 mg/day, steady-state C<sub>max</sub> values and systemic exposure for DAPA and D3OG were incrementally higher with decreasing renal function compared to patients with T2D and normal renal function. An in vitro study of isolated human kidney microsomes showed higher UGT1A9 activity and D3OG formation rate relative to human liver and intestinal microsomes (3- and 109-fold higher, respectively). Renal glucose clearance values were 53%, 77%, and 85% lower in T2D patients with mild, moderate, and severe renal impairment, respectively, compared to subjects with normal renal function. The corresponding steady-state glucose clearance values, with DAPA 20 mg/day for 7 days, were 42%, 83%, and 84% lower. Mean 24-h urinary glucose excretion and changes in fasting serum glucose were similarly reduced with increasing degrees of renal impairment. There were no marked differences in serum electrolytes, including sodium, between groups at baseline or after treatment. DAPA was well tolerated, with no discontinuations.

**Conclusions:** These findings indicate that a substantial proportion of DAPA metabolism occurs via UGT1A9 in the kidney resulting in higher systemic exposures of dapagliflozin in patients with renal impairment compared to those with normal renal function. The PD effects of DAPA are reduced as renal function decreases, despite higher systemic DAPA exposure, due to a decreased GFR and subsequent decrease in filtered glucose load. These results suggest that DAPA may have reduced efficacy in patients with moderate to severe renal dysfunction.

	Classification of renal impairment	DAPA 50 mg - single dose		DAPA 20 mg - 7 days	
		C <sub>max</sub>	AUC <sub>0-τ</sub>	C <sub>max</sub>	AUC <sub>0-τ</sub>
DAPA	Mild	14%	25%	4%	32%
	Moderate	26%	46%	6%	60%
	Severe	36%	65%	9%	87%
D3OG	Mild	20%	47%	20%	54%
	Moderate	36%	93%	37%	110%
	Severe	51%	140%	52%	169%

Data are geometric means derived from model-based linear regression of observed data values. Values represent percent increase in comparison to normal renal function for the midpoint of each renal impairment category.

Clinical Trial Registration Number: NCT00554450

Supported by: AstraZeneca and Bristol-Myers Squibb

## 750

### Measures of beta cell function and insulin sensitivity over time in patients with type 2 diabetes receiving dapagliflozin versus glipizide as add-on therapy to metformin

A. Langkilde<sup>1</sup>, K. Rohwedder<sup>2</sup>, N. Iqbal<sup>3</sup>, L. Ying<sup>3</sup>, A. Salsali<sup>3</sup>;

<sup>1</sup>AstraZeneca, Mölndal, Sweden, <sup>2</sup>AstraZeneca, Wedel, Germany, <sup>3</sup>Bristol-Myers Squibb, Princeton, USA.

**Background and aims:** Dapagliflozin (DAPA), a selective inhibitor of sodium glucose co-transporter 2 (SGLT2), reduces plasma glucose and promotes weight loss by increasing renal glucose excretion. By this mechanism, DAPA has the potential to be disease modifying and to affect the underlying pathophysiology of type 2 diabetes. Here we evaluate the effect of DAPA on  $\beta$ -cell function and insulin sensitivity in comparison to glipizide (GLIP) as add-on therapy to metformin.

**Materials and methods:** In this 52-wk study (N=814 [636 completers]) with a 52-wk extension (N=624 [432 completers]), patients with type 2 diabetes (mean baseline HbA1c = 7.7%, diabetes duration = 6.3 y) inadequately controlled with metformin (1500–2500 mg/day) received DAPA ( $\leq 10$  mg/day) or GLIP ( $\leq 20$  mg/day) as add-on therapy. Adjusted mean change from baseline (95% CI) in fasting insulin, proinsulin, C-peptide, homeostatic model assessment-2 $\beta$  (HOMA-2  $\beta$ ) (% $\beta$ ), and HOMA-2 insulin sensitivity was evaluated at wks 26, 52, 78, and 104 using longitudinal repeated measures analysis.

**Results:** The primary end point, adjusted mean change from baseline in HbA1c at week 52, was identical for DAPA and GLIP, -0.52%. At 104 wks, mean HbA1c (95% CI) was reduced more with DAPA, -0.32% (-0.42%, -0.21%) than with GLIP, -0.14% (-0.25%, -0.03%). Mean changes in fasting plasma glucose (95% CI) for DAPA and GLIP were -23.1 (-26.6, -19.9) and -19.0 (-22.3, -15.8) mg/dL at wk 52 and -20.2 (-23.8, -16.5) and -12.2 (-15.9, -8.5) mg/dL at wk 104, respectively.  $\beta$ -Cell function and insulin sensitivity were assessed by fasting insulin, proinsulin, and C-peptide levels and by HOMA-2, which has not been validated in the setting of urinary glucose loss with SGLT2 inhibitors. Fasting insulin decreased with DAPA at wk 52, -1.64 (-2.47, -0.82) and 104, -2.35 (-3.86, -0.85)  $\mu$ U/L, but increased with GLIP, 1.28 (0.44, 2.12) and 1.16 (-0.42, 2.74)  $\mu$ U/L. Proinsulin decreased at wk 52, -5.94 (-7.89, -3.99) and 104, -5.46 (-7.52, -3.39) pmol/L with DAPA and increased with GLIP, 5.32 (3.35, 7.29) and 4.74 (2.61, 6.88) pmol/L. C-peptide did not change with DAPA at wk 52, -0.08 (-0.20, 0.03) or wk 104, -0.14 (-0.28, -0.01) ng/mL, but increased with GLIP, 0.48 (-0.36, 0.59) and 0.35 (0.21, 0.49) ng/mL. HOMA-2 % $\beta$  increased more with GLIP, 28.7% (24.5%, 33.0%) than with DAPA, 15.0% (10.8%, 19.2%) at wk 52 and at wk 104, 18.0% (13.3%, 22.8%) for GLIP vs 11.6% (7.1%, 16.2%) for DAPA. GLIP decreased HOMA-2 insulin sensitivity at wk 52, -4.0% (-6.0%, -2.0%) and 104, -1.4% (-4.4%, 1.6%), whereas DAPA increased HOMA-2 insulin sensitivity at wk 52, 6.0% (4.0%, 8.0%) and 104, 6.5% (3.6%, 9.4%).

**Conclusions:** DAPA showed a greater durability of glycaemic control than GLIP and lowered fasting proinsulin. Patients receiving DAPA exhibited improvement of  $\beta$ -cell function and improvement of insulin sensitivity, while GLIP also showed improvement of  $\beta$ -cell function however worsening of insulin sensitivity.

Clinical Trial Registration Number: NCT00660907

Supported by: AstraZeneca and Bristol-Myers Squibb

## 751

### Dapagliflozin produces long-term reductions in body weight, waist circumference and total fat mass in patients with type 2 diabetes inadequately controlled on metformin

J. Bolinder<sup>1</sup>, Ö. Ljunggren<sup>2</sup>, L. Johansson<sup>2,3</sup>, J.P.H. Wilding<sup>4</sup>, A.M. Langkilde<sup>3</sup>, C.D. Sjöström<sup>3</sup>, J. Sugg<sup>5</sup>, S. Parikh<sup>5</sup>;

<sup>1</sup>Karolinska Institute, Stockholm, Sweden, <sup>2</sup>Uppsala University, Sweden, <sup>3</sup>AstraZeneca, Mölndal, Sweden, <sup>4</sup>University of Liverpool, UK, <sup>5</sup>AstraZeneca, Wilmington, USA.

**Background and aims:** Dapagliflozin, a selective inhibitor of sodium-glucose cotransporter 2 (SGLT2), reduces hyperglycaemia in patients with type 2 diabetes mellitus (T2DM) by increasing urinary glucose excretion. Weight loss has been a consistent associated finding. A previous report demonstrated reduced total body weight (TBW) at 24 weeks (dapagliflozin vs placebo difference -2.08 kg; 95% CI -2.84, -1.31), principally accounted for by reduced fat mass (dapagliflozin vs placebo difference -1.48 kg; 95% CI -2.22, -0.74). Here we investigated whether these reductions were maintained after a further 78 weeks of dapagliflozin treatment.

**Materials and methods:** This international, multi-centre, randomised, parallel-group, double-blind, placebo-controlled study enrolled patients with T2DM (mean values: women 63.3 and men 58.6 years; HbA1c 7.17%; BMI 31.9 kg/m<sup>2</sup>; and body weight 91.5 kg) inadequately controlled on metformin. A total of 182 patients were randomised 1:1 to receive dapagliflozin 10 mg/day or placebo added to open-label metformin for a 24-week double-blind treatment period followed by a 78-week site- and patient-blinded extension period. Patients received rescue therapy with sitagliptin 100 mg if their HbA1c was  $>8.0\%$  from week 24 to 50 and  $>7.5\%$  from week 50 to 102. The main objectives were to assess TBW, waist circumference and dual-energy x-ray absorptiometry total-body fat mass changes from baseline at week 102, and the proportion of patients achieving a body weight reduction of  $\geq 5\%$  at week 102. **Results:** A total of 140 (76.9%) patients completed the 102-week study. Dapagliflozin vs placebo differences were as follows: TBW, -2.42 kg (95% CI -3.64, -1.21); waist circumference, -2.1 cm (95% CI -3.9, -0.3); fat mass, -1.34 kg (95% CI -2.44, -0.23); proportion of patients achieving weight reduction of  $\geq 5\%$ , 10.6% (95% CI -1.4, 22.6). At 102 weeks, dapagliflozin maintained both urinary glucose excretion and reductions in HbA1c and fasting plasma glucose with a lower proportion of patients requiring rescue therapy or discontinuing due to poor glycaemic control (Table). By 102 weeks, in the dapagliflozin vs placebo groups, respectively, adverse events were reported in 71.4 vs 69.2%; events of vulvovaginitis, balanitis, and related genital infection in 2.2 vs 1.1%; events of urinary tract infection in 6.6 vs 7.7%; and hypoglycaemic episodes in 4.4 vs 5.4%.

**Conclusion:** Dapagliflozin produces long-term reductions in TBW, fat mass and waist circumference in the context of sustained improvements in glycaemic control compared with placebo in T2DM patients inadequately controlled with metformin. On-going caloric loss from increased urinary glucose excretion with dapagliflozin is likely to have accounted for these long-term changes in TBW and fat mass.

Changes in body composition, glycaemia and glucosuria with dapagliflozin over 102 weeks

	Placebo + Metformin (N/n = 91/71)	Dapagliflozin 10mg + Metformin (N/n = 89/69)
<b>Total body weight, kg</b>		
Baseline mean (SD)	90.91 (13.73)	92.06 (14.13)
Change at week 102*	-2.12 (-2.97, -1.27)	-4.54 (-5.43, -3.66)
Difference vs placebo		-2.42 (-3.64, -1.21)
<b>Waist circumference, cm</b>		
Baseline mean (SD)	104.5 (12.3)	105.6 (10.1)
Change at week 102*	-2.9 (-4.1, -1.6)	-5.0 (-6.3, -3.6)
Difference vs placebo		-2.1 (-3.9, -0.3)
<b>Total body fat mass, kg</b>		
Baseline mean (SD)	33.40 (9.73)	33.72 (8.63)
Change at week 102*	-1.46 (-2.25, -0.68)	-2.80 (-3.67, -1.93)
Difference vs placebo		-1.34 (-2.44, -0.23)
<b>Weight loss <math>\geq 5\%</math></b>		
Proportion at week 102†	16.5% (8.9, 24.1)	27.1% (17.8, 36.4)
Difference vs placebo		10.6% (-1.4, 22.6)
<b>HbA1c, %</b>		
Baseline mean (SD)	7.16 (0.53)	7.19 (0.44)
Change at week 102*‡	0.12 (-0.01, 0.27)	-0.30 (-0.43, -0.16)
Difference vs placebo		-0.42 (-0.62, -0.22)
<b>FPG, mmol/L</b>		
Baseline mean (SD)	8.30 (1.39)	8.21 (1.37)
Change at week 102*‡	-0.48 (-0.73, -0.28)	-1.28 (-1.55, -1.02)
Difference vs placebo		-0.80 (-1.16, -0.44)
<b>Rescued§</b>		
Proportion at week 102†	33.3% (24.1, 42.5)	13.5% (6.4, 20.7)
Difference vs placebo		-19.7% (-31.7, -7.8)
<b>Urine glucose, mmol/L§</b>		
Baseline mean (SD)	1.25 (3.56)	1.72 (6.70)
Change at week 102	-0.11 (-0.76, 0.54)	115.0 (96.0, 134.0)

N = the number of patients in the full analysis set. n = the number of patients completing the study. FPG, fasting plasma glucose. \*Data are adjusted mean changes from baseline and 95% CI derived from a mixed model. †Data are adjusted proportions and 95% CI derived from logistic regression. ‡Analysis excludes data after initiation of rescue therapy. §Rescued or discontinued due to poor glycaemic control. ¶Spot urine glucose in the fasting state. ||Data are unadjusted mean changes from baseline and 95% CI.

Clinical Trial Registration Number: NCT00855166

Supported by: AZ and BMS

## 752

**Dapagliflozin corrects plasma glucose levels after acute and chronic treatment: a mechanistic examination of glucose fluxes and transcription profiles**

B. Zinker, X. Ma, H. Liu, C. Cai, R. Ponticello, J. Zalaznick, B. Guan, W.-P. Yang, V. Patel, A. He, J. Chen, J. Whaley; Bristol-Myers Squibb, Princeton, USA.

**Background and aims:** Dapagliflozin (Dapa) is a potent ( $K_i=0.55$  nM), selective SGLT2 inhibitor (>1400-fold vs SGLT1) which reduces renal glucose reabsorption and may provide an insulin-independent mechanism for type 2 diabetes treatment. Dapa reduces hyperglycemia acutely and prevents progression to the diabetic state during chronic dosing in male ZDF rats without hypoglycemia. To examine the impact of Dapa treatment on  $\beta$ -cell insulin content and function, as well as endogenous glucose production and disposal, prediabetic male ZDF rats and their lean littermates were used in acute and chronic studies. Liver, adipose, skeletal muscle and kidney gene transcription profiles were also evaluated following chronic treatment.

**Materials and methods:** Male ZDF and lean rats at 11 wk of age were dosed acutely with vehicle (Vh) or Dapa at 0.5 or 1.0 mg/kg, and studied over a 2 hr period in which whole body glucose fluxes were examined. For the chronic study rats began treatment (*q.d.*, *p.o.*) prior to frank diabetes (6 wk of age) which continued to 11 wk of age with either Vh (lean and male ZDF) or 0.5 mg/kg Dapa (male ZDF). At 48 hr after the last dose, a euglycemic-hyperinsulinemic clamp was performed and tissues were harvested for mRNA expression.

**Results:** Acute Arterial plasma glucose levels decreased (30 min) and stabilized (90 min) in male ZDF rats following both Dapa doses, while Vh and Lean Dapa glucose levels were unchanged. In lean rats Dapa was associated with a dose-dependent increase in glucose production (rate of appearance [Ra];  $9.8 \pm 0.5$  vs  $6.5 \pm 0.4$  mg/kg-min, 1.0 mg/kg vs Vh,  $p < 0.001$ ). By comparison, in male ZDF rats 1.0 mg/kg was associated with increased Ra ( $13.5 \pm 0.6$  mg/kg-min;  $p < 0.01$ ) vs Vh. Dapa was associated with decreased glucose utilization (GUR) in Lean at 0.5 mg/kg, and diminished GUR in a non-dose-dependent manner in ZDF rats vs Vh due to urine glucose loss. Chronic: Dapa maintained plasma glucose and  $HbA_{1c}$  to levels near those in Vh Lean, and decreased basal glucose flux vs that seen in the ZDF Vh ( $6.6 \pm 0.4$  vs  $9.3 \pm 0.8$  mg/kg-min,  $p = 0.01$ ). Pancreatic insulin content was maintained at baseline levels, and plasma insulin levels were increased, with Dapa vs the declines in ZDF Vh, respectively, after 5 wks. During the clamp, whole body glucose disposal and glucose uptake in epididymal fat and soleus muscle were similar in the Dapa and Vh ZDF groups. Dapa increased glucose infusion rate ( $8.8 \pm 0.7$  vs  $3.8 \pm 0.6$  mg/kg-min,  $p < 0.05$ ), and decreased endogenous glucose production on an absolute ( $3.6 \pm 0.5$  vs  $10.7 \pm 2.1$  mg/kg-min,  $p < 0.001$ ) and relative basis vs ZDF Vh ( $-45 \pm 9\%$  vs  $20 \pm 28\%$ ,  $p < 0.05$ ). Transcription profiling revealed liver to have the greatest changes in gene expression; Dapa treatment resulted in significant increases in SCD1, GCK, INHBC mRNA and decrease in HSD11b1 mRNA. No change in expression of renal glucose transporters was observed in the kidney suggesting there is little renal glucose transporter transcriptional compensation for SGLT2 inhibition in this model. No significant enhancement of cellular proliferation or tumor promotor-related expression was observed in any tissue examined.

**Conclusion:** Acute Dapa treatment leads to dose-dependent increases in endogenous glucose production and decreases plasma glucose in hyperglycemic rats without hypoglycemia.

Supported by: BMS and AZ

## 753

**Long-term effectiveness of dapagliflozin over 104 weeks in patients with type 2 diabetes poorly controlled with insulin**

K. Rohwedder<sup>1</sup>, J.P.H. Wilding<sup>2</sup>, V. Woo<sup>3</sup>, J. Sugg<sup>4</sup>, S. Parikh<sup>4</sup>;

<sup>1</sup>AstraZeneca, Wedel, Germany, <sup>2</sup>University of Liverpool, UK, <sup>3</sup>University of Manitoba, Winnipeg, Canada, <sup>4</sup>AstraZeneca, Wilmington, USA.

**Background and aims:** Dapagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, increases urinary glucose excretion and reduces hyperglycaemia in type 2 diabetes (T2DM) independent of insulin secretion or action.

**Materials and methods:** We report results after 104 weeks of patient- and centre-blinded treatment in patients with T2DM poorly controlled on insulin ( $n=808$ ; mean baseline  $HbA_{1c}$  8.53%) randomised to receive placebo, or dapagliflozin 2.5, 5 or 10 mg/day added to background insulin (mean baseline insulin 77 IU/day)  $\pm$  oral glucose-lowering drugs. Analyses at 24 weeks

(primary endpoint) and at 48 weeks were reported previously. At 48 weeks, patients on dapagliflozin 5 mg were switched to 10 mg (5/10-mg group). Insulin was uptitrated if  $HbA_{1c}$  was  $>7.5\%$  from 52 to 65 weeks or  $>7.0\%$  from 78 to 104 weeks. 63.6% of patients completed 104 weeks. Analyses over 104 weeks used observed cases and included data after insulin uptitration.

**Results:** Mean  $HbA_{1c}$  changes from baseline at 104 weeks were  $-0.43\%$  in the placebo group and  $-0.64\%$  to  $-0.82\%$  in the dapagliflozin groups (corresponding  $HbA_{1c}$  values excluding data after insulin uptitration were  $-0.06\%$  vs  $-0.49\%$  to  $-0.71\%$ ). In the placebo group, mean insulin dose increased by 18.3 IU/day and weight increased by 1.8 kg at 104 weeks, whereas in the dapagliflozin groups, insulin dose was stable and weight decreased by 0.9 to 1.4 kg (weight values excluding data after insulin uptitration were  $+0.91$  kg in the placebo group vs  $-1.47$  to  $-1.97$  kg in the dapagliflozin groups). Adverse events, including hypoglycaemia, were balanced across groups (Table). Proportions of patients with events suggestive of genital infection and of urinary tract infection (UTI) were higher with dapagliflozin vs placebo (genital infection 7.4 to 14.3% vs 3.0%; UTI 8.4 to 13.8% vs 5.6%) but most occurred in the first 24 weeks and most were single episodes that responded to routine management.

**Conclusion:** Dapagliflozin treatment over 104 weeks in patients with T2DM poorly controlled on insulin was well tolerated and resulted in reductions in  $HbA_{1c}$  and weight without an increase in mean daily insulin requirements.

Efficacy and safety of dapagliflozin at 104 weeks in patients with T2DM poorly controlled on insulin

	PLA + INS	DAPA 2.5mg + INS	DAPA 5/10mg + INS	DAPA 10mg + INS
N	.	.	.	.
Randomised	197	203	212	196
Completing	108	134	129	142
<b>HbA<sub>1c</sub>, %</b>	.	.	.	.
Baseline mean	8.47	8.46	8.62	8.58
w104 change*	-0.43	-0.64	-0.82	-0.79
(95%CI)	(-0.58,-0.28)	(-0.78,-0.50)	(-0.96,-0.68)	(-0.92,-0.65)
Diff. vs PLA	.	-0.21	-0.39	-0.35
(95%CI)	.	(-0.41,-0.01)	(-0.59,-0.18)	(-0.56,-0.15)
<b>Uptitration†</b>	.	.	.	.
Proportion‡	50.4%	29.1%	26.5%	25.5%
(95%CI)	(43.5,57.2)	(22.9,35.3)	(20.5,32.4)	(19.4,31.6)
Diff. vs PLA	.	-21.3%	-23.9%	-24.9%
(95%CI)	.	(-30.5,-12.0)	(-33.0,-14.8)	(-34.1,-15.6)
<b>INS dose, IU/d</b>	.	.	.	.
Baseline mean	74.0	79.9	77.1	78.0
w104 change*	18.3	4.1	1.6	-0.8
(95%CI)	(13.7,22.9)	(-0.2,8.4)	(-2.7,5.9)	(-5.1,3.5)
Diff. vs PLA	.	-14.3	-16.8	-19.2
(95%CI)	.	(-20.5,-8.0)	(-23.1,-10.5)	(-25.5,-12.9)
<b>Weight, kg</b>	.	.	.	.
Baseline mean	94.5	93.0	93.4	94.6
w104 change*	1.8	-0.9	-1.0	-1.4
(95%CI)	(1.0,2.6)	(-1.6,-0.2)	(-1.7,-0.2)	(-2.1,-0.7)
Diff. vs PLA	.	-2.7	-2.7	-3.2
(95%CI)	.	(-3.8,-1.7)	(-3.8,-1.7)	(-4.2,-2.1)
<b>AEs</b>	.	.	.	.
Total	78.2%	80.2%	78.3%	80.1%
Serious	19.8%	19.3%	15.1%	18.4%
Discontinuation	6.6%	5.0%	9.4%	5.6%
Deaths	0	0	0.9%	0.5%
<b>Hypoglycaemia</b>	.	.	.	.
Total	61.9%	69.3%	61.3%	60.7%
Major§	1.0%	2.0%	1.4%	1.5%

\*Data are adjusted mean change from baseline derived from mixed models. †Insulin uptitration or discontinuation due to lack of glycaemic control. ‡Data are adjusted proportions derived from logistic regression. §Defined as a symptomatic episode requiring external assistance due to severe impairment in consciousness or behaviour with a capillary or plasma glucose value  $<3$  mmol/L and prompt recovery after glucose or glucagon administration. AEs, adverse events; DAPA, dapagliflozin; INS, insulin; PLA, placebo.

Clinical Trial Registration Number: NCT00673231

Supported by: AZ and BMS



## 754

**Glycaemic and weight-reduction benefits with dapagliflozin in patients with type 2 diabetes mellitus: pooled subgroup analysis of nine clinical trials**T.A. Mansfield<sup>1</sup>, T. de Bruin<sup>2</sup>, E. Hardy<sup>2</sup>, A. Salsali<sup>3</sup>, L. Wei<sup>3</sup>, C. Wessman<sup>4</sup>, S. Parikh<sup>2</sup>;<sup>1</sup>Bristol-Myers Squibb, Princeton, USA, <sup>2</sup>AstraZeneca, Wilmington, USA, <sup>3</sup>Research & Development, Bristol-Myers Squibb, Princeton, USA,<sup>4</sup>AstraZeneca, Mölndal, Sweden.

**Background and aims:** Reduction of glycaemic levels and body weight are fundamental goals in management of patients with type 2 diabetes mellitus (T2DM). Dapagliflozin is a selective inhibitor of sodium glucose cotransporter 2 (SGLT2) that reduces plasma glucose independently of insulin secretion or action by increasing the urinary excretion of excess glucose. This report analyzed the effect of dapagliflozin on HbA1c and weight from data pooled from nine double-blind, randomized clinical trials of dapagliflozin in patients with T2DM to determine if improvements in these outcomes are dependent on baseline parameters.

**Materials and methods:** Patients with T2DM (N=4047) received daily doses of dapagliflozin 2.5 mg, 5 mg, or 10 mg or placebo for 24 weeks as monotherapy; as add-on to metformin, glimepiride, pioglitazone, or insulin; or as initial combination with metformin. Adjusted mean change from baseline in HbA1c, excluding data after rescue (LOCF), was analyzed by an ANCOVA model with treatment group, subgroup, and study as categorical factors; and interaction between treatment group and subgroup, baseline HbA1c and weight, and study-by-baseline interaction with HbA1c and weight as continuous covariates. Treatment by subgroup interactions were analyzed for baseline HbA1c, estimated glomerular filtration rate (eGFR), age, gender, race, ethnicity (US only), geographic region, BMI, and T2DM duration. *P* values are reported for subgroup interaction based on average treatment effect relative to placebo; a *P* value <0.1 indicated a potential interaction.

**Results:** Control-corrected mean changes from baseline in the individual studies for HbA1c in the dapagliflozin treatment groups (range, %) were: monotherapy -0.35 to -0.84, add-on metformin -0.28 to -0.54, add-on glimepiride -0.44 to -0.68, add-on pioglitazone -0.40 to -0.55, add-on insulin -0.45 to -0.60, and initial combination with metformin -0.54 to -0.70. No interaction of change from baseline in HbA1c was detected for gender, race, ethnicity, region, BMI, or T2DM duration. Potential subgroup interactions were detected for baseline HbA1c (*P*<0.0001), eGFR category (*P*=0.015), and age (*P*=0.054). After controlling for eGFR, age was no longer a factor affecting efficacy. In the analysis of body weight, placebo-corrected change from baseline ranged from -0.46 to -2.16 kg in the overall population. No subgroup interactions of weight reduction with age, gender, baseline HbA1c, BMI, eGFR, or duration of T2DM were detected; potential subgroup interactions were detected for geographic region (*P*=0.03), race (*P*=0.06), and ethnicity (*P*=0.09). **Conclusion:** Dapagliflozin provides consistent reductions in HbA1c and body weight across a range of subgroups of patients with T2DM. The magnitude of the reduction in HbA1c is influenced by baseline HbA1c and eGFR, as expected given the dependence of the MOA of dapagliflozin on the renal filtration of glucose. Additional benefits in body weight reduction appear to be independent of age, gender, baseline HbA1c, BMI, eGFR, and duration of diabetes.

*Clinical Trial Registration Number:* NCT00528372, NCT00736879, NCT00528879, NCT00855166, NCT00680745, NCT00683878, NCT00673231, NCT00859898, NCT00643851

*Supported by:* BMS, AstraZeneca

## 755

**Dapagliflozin is more effective than glipizide in achieving the composite outcome of glycaemic control, weight reduction and lack of hypoglycaemia**G.D. Wygant<sup>1</sup>, A.-G. Chalamandaris<sup>2</sup>, U.H. Illoeje<sup>3</sup>, A. Salsali<sup>1</sup>, S. Grandy<sup>4</sup>, J.E. Sugg<sup>4</sup>, K. Rohwedder<sup>5</sup>, S.J. Parikh<sup>4</sup>;<sup>1</sup>Bristol-Myers Squibb, Princeton, USA, <sup>2</sup>Bristol-Myers Squibb, Braine-l'Alleud, Belgium, <sup>3</sup>Bristol-Myers Squibb, Wallingford, USA, <sup>4</sup>AstraZeneca, Wilmington, USA, <sup>5</sup>AstraZeneca, Wedel, Germany.

**Background and aims:** Management of diabetes often results in trade-offs between glycaemic control and untoward effects of weight gain and hypoglycaemic events. Dapagliflozin (DAPA), a selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, reduces hyperglycaemia and body weight with

a low risk of hypoglycaemia by increasing urinary glucose excretion in an insulin-independent manner. Key results of a randomised, double-blind trial of DAPA (up to 10 mg/d, n=400) vs glipizide (GLIP, up to 20 mg/d, n=401) as add-on therapies to metformin (MET, median 2000 mg/d) in subjects with T2DM inadequately controlled on MET (mean baseline HbA1c 7.72%), have been published elsewhere. Overall, 77.9% of randomised patients (322 in the DAPA group and 314 in the GLIP group) completed the study. This post-hoc analysis assessed the proportion of subjects achieving the clinically relevant composite endpoint of HbA1c <7%, no major or minor hypoglycaemic (hypo) events and weight loss ≥ 3%.

**Materials and methods:** Proportion with HbA1c <7% at 52 weeks (LOCF) was evaluated; subjects with HbA1c <7% at baseline were excluded (21% in DAPA + MET [84/400] arm and 19% in GLIP + MET [78/401] arm). Major/minor hypo events included those with onset on/after first day of double blind treatment and on/prior to last day of short-term treatment + 4 days. Proportion of patients with total body weight reduction of ≥ 3% from baseline to week 52 LOCF was calculated. Confidence intervals (CI) for the difference between study arms were calculated using the exact method.

**Results:** At 52 weeks, the composite endpoint was achieved by 20.6% of subjects in the DAPA + MET arm (65/316) versus 1.9% in the GLIP + MET (6/323) arm. The difference of DAPA + MET versus GLIP + MET was 18.7% (95% CI: 14.3%, 23.8%).

**Conclusion:** More subjects treated with DAPA + MET achieved the composite endpoint of HbA1c <7%, no major or minor hypoglycaemic events and ≥ 3% weight loss at 1 year compared with subjects treated with GLIP + MET. These results demonstrate that DAPA, with its insulin-independent mechanism of action of increased urinary glucose excretion, can positively impact key treatment parameters while avoiding hypoglycaemia. \*Affiliation at the time of writing

*Clinical Trial Registration Number:* NCT00660907

*Supported by:* BMS and AZ

## 756

**Dapagliflozin treatment for type 2 diabetes mellitus patients with a history of cardiovascular disease**L. Leiter<sup>1</sup>, W. Cefalu<sup>2</sup>, T. DeBruin<sup>3</sup>, I. Gause-Nilsson<sup>4</sup>, J. Sugg<sup>3</sup>, S. Parikh<sup>3</sup>;<sup>1</sup>University of Toronto, Canada, <sup>2</sup>University of Louisiana, Baton Rouge, USA,<sup>3</sup>AstraZeneca, Wilmington, USA, <sup>4</sup>AstraZeneca, Mölndal, Sweden.

**Background and aims:** Dapagliflozin (DAPA) is a selective SGLT2 inhibitor with glucuretic effects under investigation for the treatment of type 2 diabetes mellitus (T2DM). DAPA-induced urinary glucose excretion results in improved glycaemic control and body weight (BW) loss. Here we present the results of 1 of 2 phase 3 trials conducted to assess DAPA efficacy and safety in patients with T2DM and documented cardiovascular disease (CVD).

**Materials and methods:** Patients (N=964) with HbA1c ≥7.0%–≤10.0%, were randomised to receive double-blind DAPA 10 mg/d or placebo (PBO) for 24 wks (2 extensions ongoing adding up to 80 wks) and stratified by age (<65 or ≥65 y), insulin (INS) use, and time from most recent qualifying CV event. INS doses were reduced by 25% at randomisation. The co-primary end points were change from baseline (BL) in HbA1c and the proportion of patients achieving a 3-item composite end point: reduction of ≥0.5% in HbA1c, ≥3% in BW, and ≥3 mmHg in seated systolic blood pressure (SBP).

**Results:** The mean age was 64 y, and 47% were ≥65 y. Mean T2DM duration was 13 y. Most patients reported the use of 1–2 oral anti-hyperglycaemic agents. Twenty percent received only INS, and an additional 41% used INS in combination with other treatments. A history of hypertension was present in 93% of patients. Reductions from BL were greater for DAPA vs PBO in HbA1c (BL 8.1%) and SBP (BL 134.7 mmHg) (Table). More DAPA patients achieved the 3-item end point vs PBO. BW (BL 93.9 kg) was significantly reduced for DAPA vs PBO, and more patients in the DAPA group achieved a ≥3% decrease in BW. In patients with BL body mass index ≥27 kg/m<sup>2</sup> (88%), a greater proportion achieved weight loss of ≥5% in the DAPA vs PBO group (18.4% vs 4.8%, *P*<0.0001). In those receiving INS, the mean daily INS dose increased 10% (5.3 IU/d) with PBO vs no change with DAPA (-0.0 IU/d, nominal *P*<0.05). Stratified analyses yielded similar results to the overall analysis; however, SBP reduction was not significantly different at 24 wks in patients ≥65 y. Adverse events (AEs) and serious AEs were balanced between groups, but more patients on DAPA had events suggestive of vulvovaginitis/balanitis or urinary tract infection. Hypoglycaemia was observed more often in those patients on INS at randomisation and for a slightly higher proportion of patients in the DAPA group vs the PBO group (21.0% vs 17.4%). Unadjusted adverse CV events (DAPA 4.4% vs PBO 5.2%) and serious CV

events (DAPA 2.7% vs PBO 2.3%) were reported for similar proportions of patients in both groups.

**Conclusion:** When added to standard of care, DAPA improved glycaemic control with a low incremental risk of hypoglycaemia, was associated with BW loss and lowering of BP, and was well tolerated in an older, high CV risk population with advanced T2DM and comorbid CVD. The BW loss accompanying DAPA treatment suggests an added health benefit beyond glycaemic control for a predominantly overweight population.

	PBO N=482	DAPA 10 mg N=480
HbA1c, % <sup>a</sup>	0.07 (0.04)	-0.33 (0.04)*
3-item composite end point,% <sup>b</sup>	1.9%	10.0%*
BW, % change <sup>a</sup>	-0.61 (0.18)	-2.53 (0.17)*
SBP, 8 wks, mmHg <sup>a</sup>	0.86 (0.71)	-1.85 (0.71)†
SBP, 24 wks, mmHg <sup>a</sup>	0.32 (0.71)	-2.70 (0.71)‡

<sup>a</sup>Data are adjusted mean change from BL (SE), last observation carried forward. <sup>b</sup>Data are adjusted proportion, last observation carried forward. \*P<0.0001; †P=0.0007; ‡P=0.0002 vs PBO.

Clinical Trial Registration Number: NCT01042977

Supported by: AstraZeneca and Bristol-Myers Squibb

## 757

### Dapagliflozin pharmacokinetic and pharmacodynamic relationships in patients with type 2 diabetes and healthy subjects: a meta-analysis of 9 clinical studies

D.W. Boulton, X. Liu, M. Hesney, S.C. Griffen, F.P. LaCreta, S. Kasichayanula; Bristol-Myers Squibb, Princeton, USA.

**Background and aims:** Dapagliflozin (DAPA), a potent, highly-selective, orally active inhibitor of the human renal sodium glucose co-transporter (SGLT2), is being developed for the treatment of type 2 diabetes mellitus (T2DM).

**Materials and methods:** DAPA pharmacokinetic (PK) parameters and pharmacodynamic (PD) endpoints (renal glucose clearance (CL<sub>Rgl</sub>) and 24-h urinary glucose amount (Ae<sub>gl</sub>) were pooled from 9 Clinical Pharmacology studies in subjects with normal renal function (n=270; 179 healthy subjects (HS) and 81 patients with T2DM). To assess the proportionality of DAPA PK parameters (C<sub>max</sub> and AUC) to dose, linear regression analyses of log(C<sub>max</sub>) on log(dose) and of log(AUC) on log(dose) were performed using a power model. A sigmoid maximum effect (E<sub>max</sub>) model was used to assess the relationship between DAPA dose or exposure and urinary PD including change from baseline in 24-hr Ae<sub>gl</sub> and CL<sub>Rgl</sub>.

**Results:** DAPA C<sub>max</sub> and AUC values increased proportionally to DAPA dose from 0.1 mg (lowest dose with definable PK) up to 500 mg (highest dose studied), and no clear differences were observed between HS and patients with T2DM. Dose-related increases in 24 h Ae<sub>gl</sub> was observed following the administration of DAPA doses ≥ 0.3 mg, and maximal increases in Ae<sub>gl</sub> were observed at doses ≥ 20 mg/day. Patients with T2DM had greater Ae<sub>gl</sub> compared to HS (E<sub>max</sub>: 83 and 66 g/24 h, respectively, and the doses for which one-half of E<sub>max</sub> was predicted (ED<sub>50</sub>) were 4.0 and 4.7 mg for healthy subjects and patients with T2DM, respectively). CL<sub>Rgl</sub> was not different between HS and patients with T2DM.

**Conclusion:** DAPA PK were linear over a wide dose range (0.3–500 mg) and were not different between HS, and patients with T2DM. The Ae<sub>gl</sub> was higher in patients with T2DM compared to HS due to their higher plasma or serum glucose concentrations, resulting in a greater amount of glucose being renally filtered. CL<sub>Rgl</sub> was not different between these populations.

Supported by: BMS and AZ

## 758

### Dapagliflozin is safe and effective as add on therapy to sitagliptin with or without background metformin

S. Jabbour<sup>1</sup>, E. Hardy<sup>2</sup>, J. Sugg<sup>2</sup>, S. Parikh<sup>2</sup>;

<sup>1</sup>Jefferson Medical College of Thomas Jefferson University, Philadelphia,

<sup>2</sup>AstraZeneca, Wilmington, USA.

**Background and aims:** Dapagliflozin is a selective sodium glucose co-transporter 2 (SGLT2) inhibitor in development for the treatment of type 2 diabetes.

Dapagliflozin reduces hyperglycaemia through the inhibition of filtered glucose reabsorption resulting in an increase in urinary glucose excretion.

**Materials and methods:** In this 24-week, randomised, double-blind, placebo controlled study with a 24-week blinded extension period, 452 patients with inadequate glycaemic control on a stable DPP-4 inhibitor with or without metformin received dapagliflozin 10 mg QD or placebo plus sitagliptin (100mg QD: Stratum 1) or sitagliptin plus metformin (≥ 1500 mg QD: Stratum 2).

**Results:** Baseline (BL) HbA1c, fasting plasma glucose (FPG) and body weight values for placebo and dapagliflozin were 7.97% and 7.90%, 9.06 mmol/L [163mg/dL] and 9.00 mmol/L [162 mg/dL], and 89 kg and 91 kg respectively. At week 24 significant improvements were observed with dapagliflozin versus placebo (LOCF, P<0.0001) in each of the 3 variables. Whether the data after rescue were excluded or included, glycaemic and weight benefits that were observed at week 24 were maintained through week 48 in the overall data set and in each stratum (Table shows values excluding rescue). Over 48 weeks 31.8% of subjects receiving dapagliflozin were discontinued or rescued for failing to achieve glycaemic targets compared to 56.6% with placebo. Adverse events (AEs) were slightly more frequent in the dapagliflozin group (24 weeks: placebo - 109 subjects [48.2%], dapagliflozin - 119 subjects [52.9%]; 48 weeks: placebo - 138 [61.1%], dapagliflozin - 149 [66.2%]); discontinuations as a result of AEs were infrequent and balanced (24 weeks: placebo - 5 [2.2%]; dapagliflozin - 7 [3.1%]; 48 weeks: placebo - 7 [3.1%]; dapagliflozin - 7 [3.1%]). At week 24, events of genital infections and urinary tract infection were more frequent with dapagliflozin (9.3% and 5.8%) than with placebo (0.4% and 3.5%).

**Conclusion:** Dapagliflozin is safe and effective in lowering blood glucose and body weight in patients inadequately controlled with sitagliptin with or without metformin at Week 24 and efficacy was maintained over 48 weeks.

Placebo corrected change with Dapagliflozin 10mg/day (Excluding data after rescue)*				
Background therapy		Dapagliflozin overall	Stratum 1: Sitagliptin monotherapy	Stratum 2: Sitagliptin + metformin
		Placebo N=224 Dapagliflozin N=223	Placebo N=111 Dapagliflozin N=110	Placebo N=113 Dapagliflozin N=113
HbA1c % [95% CI]	24 weeks	-0.46 [-0.63, -0.29]	-0.55 [-0.84, -0.26]	-0.39 [-0.58, -0.19]
	48 weeks	-0.68 [-0.88, -0.48]	-0.85 [-1.25, -0.45]	-0.59 [-0.81, -0.36]
FPG mmol/L [95% CI] mg/dL [95% CI]	24 weeks	-1.29 [-1.64, -0.97] -23.4 [-29.5, -17.4]	-1.33 [-1.79, -0.88] -24.0 [-32.3, -15.8]	-1.27 [-1.76, -0.78] -22.9 [-31.7, -14.0]
	48 weeks	-1.84 [-2.31, -1.37] -33.2 [-41.6, -24.7]	-2.22 [-3.11, -1.32] -39.9 [-56.0, -23.7]	-1.67 [-2.19, -1.14] -30.0 [-39.4, -20.6]
Body weight kg [95% CI]	24 weeks	-2.03 [-2.61, -1.46]	-1.86 [-2.63, -1.08]	-2.04 [-2.90, -1.18]
	48 weeks	-2.22 [-2.99, -1.45]	-2.23 [-3.30, -1.15]	-2.07 [-3.19, -0.95]

\*Longitudinal repeated measures analysis.

CI - Confidence Interval

Clinical Trial Registration Number: NCT00984867

Supported by: AZ and BMS

## PS 058 SGLT-2 III

759

### Canagliflozin, a sodium glucose co-transporter 2 inhibitor, improves glycaemic control and is well tolerated in type 2 diabetes subjects with moderate renal impairment

J.-F. Yale<sup>1</sup>, G. Bakris<sup>2</sup>, E. Wajsb<sup>3</sup>, L. Xi<sup>4</sup>, K. Figueroa<sup>5</sup>, K. Usiskin<sup>5</sup>, G. Meininger<sup>5</sup>;<sup>1</sup>Royal Victoria Hospital and McGill University, Montreal, Canada,<sup>2</sup>University of Chicago Pritzker School of Medicine, Chicago, USA, <sup>3</sup>Janssen Research & Development, Beerse, Belgium, <sup>4</sup>Janssen Biotech, Inc, Spring House, USA, <sup>5</sup>Janssen Research & Development, L.L.C., Raritan, USA.

**Background and aims:** Canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor, lowers plasma glucose by increasing urinary glucose excretion. This randomised, double-blind, placebo (PBO)-controlled, Phase 3 study evaluated the efficacy and safety of CANA in subjects with type 2 diabetes mellitus (T2DM) and moderate renal impairment.

**Materials and methods:** Subjects with T2DM (N = 269) who had moderate renal impairment (estimated glomerular filtration rate [eGFR])  $\geq 30$  and  $< 50$  mL/min/1.73 m<sup>2</sup> received CANA 100 or 300 mg or PBO once daily. Efficacy endpoints were assessed at Week 26 and adverse events (AEs) were recorded throughout the study. Renal safety parameters were also evaluated.

**Results:** Mean baseline characteristics were similar across groups (age, 68.5 years; HbA<sub>1c</sub>, 8.0%; body weight, 91.2 kg; body mass index, 33.0 kg/m<sup>2</sup>; eGFR, 39.4 mL/min/1.73 m<sup>2</sup>); median baseline albumin/creatinine ratio (ACR) was 30.0 µg/mg. 74.0% of subjects were on insulin and 31.2% were on a sulphonylurea. As shown in the Table, HbA<sub>1c</sub> was significantly decreased at Week 26 with CANA 100 and 300 mg compared with PBO ( $P < 0.05$  for both). Both CANA doses showed numerical improvements in fasting plasma glucose (FPG) and reductions in body weight and systolic blood pressure (BP) compared with PBO. More PBO-treated subjects received rescue therapy (14.4%) than with CANA 100 or 300 mg (4.4% and 3.4%, respectively).

**Table. Summary of Efficacy Endpoints at Week 26 (LOCF)**

Parameter, mean (SE) <sup>a,b</sup>	CANA 100 mg (N = 90)	CANA 300 mg (N = 89)	PBO (N = 90)
HbA <sub>1c</sub> change, %	-0.33 (0.09)	-0.44 (0.09)	-0.03 (0.09)
Difference vs PBO	-0.30 (0.12) <sup>c</sup>	-0.40 (0.12) <sup>c</sup>	
FPG change, mmol/L	-0.8 (0.3)	-0.7 (0.3)	0.03 (0.28)
Difference vs PBO	-0.9 (0.4)	-0.7 (0.4) <sup>*</sup>	
Body weight % change	-1.2 (0.3)	-1.5 (0.3)	0.3 (0.3)
Difference vs PBO	-1.6 (0.4)	-1.8 (0.4)	
Systolic BP change, mmHg	-6.1 (1.5)	-6.4 (1.5)	-0.3 (1.5)
Difference vs PBO	-5.7 (1.9)	-6.1 (2.0)	
Diastolic BP change, mmHg	-2.6 (0.9)	-3.5 (0.9)	-1.4 (0.9)
Difference vs PBO	-1.2 (1.1)	-2.1 (1.1)	
Triglycerides % change	6.2 (4.6)	11.9 (4.6)	7.9 (4.8)
Difference vs PBO	-1.7 (6.2)	3.9 (6.1)	
LDL-C % change	6.4 (3.5)	-1.0 (3.4)	6.3 (3.6)
Difference vs PBO	0.1 (4.6)	-7.2 (4.6)	
HDL-C % change	4.0 (1.7)	3.0 (1.7)	1.5 (1.8)
Difference vs PBO	2.5 (2.3)	1.5 (2.3)	
LDL-C/HDL-C % change	4.7 (3.7)	-4.3 (3.7)	4.7 (3.8)
Difference vs PBO	0.0 (4.9)	-8.9 (4.9)	

LOCF, last observation carried forward; SE, standard error; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ANCOVA, analysis of covariance; NS, not significant. <sup>a</sup>Least squares mean (SE) change from baseline using ANCOVA; <sup>b</sup>P values are reported for pre-specified comparisons only; <sup>c</sup> $P < 0.05$  vs PBO; <sup>d</sup> $P < 0.001$  vs PBO; <sup>e</sup> $P = NS$  vs PBO; additional testing was not performed due to multiplicity control.

The overall incidence of AEs was slightly higher for CANA 100 and 300 mg than for PBO (77.8% and 74.2% vs 73.3%); serious AE rates were 11.1%, 11.2%, and 17.8%, respectively. AE-related discontinuation rates were low across groups. Incidences of superficial genital fungal infections were higher with CANA 100 and 300 mg than PBO (women, 6.3% and 4.9% vs 0%; men, 1.7% and 2.1% vs 0%). CANA 300 mg showed slightly higher urinary tract infection rates (7.9%) than other groups (5.6% for CANA 100 mg and PBO); no increases in upper tract infections were seen with CANA. Incidences of osmotic diuresis- and volume-related AEs were higher with CANA than with PBO ( $< 5\%$  per specific AE except hypotension [6.7%] and dizziness [5.6%] for CANA 300 mg). More subjects had  $\geq 1$  hypoglycaemia episode with CANA 100 and 300 mg than PBO (52.9% and 51.2% vs 36.4%). CANA 100 and 300 mg showed increases in serum creatinine (9% and 10% vs 4%) and blood urea nitrogen (9% and 6% vs 2%) compared with PBO. Changes in eGFR for CANA and PBO were approximately -10% (both doses) and -3%, respectively. Median ACR was reduced with CANA 100 and 300 mg compared with PBO (-21.6% and -12.4% vs -2.5%).

**Conclusion:** CANA was well tolerated and demonstrated efficacy in improving glycaemic control and reducing body weight in subjects with T2DM who had moderate renal impairment.

Clinical Trial Registration Number: NCT01064414

Supported by: Janssen Research & Development, L.L.C.

760

### Canagliflozin, a sodium glucose co-transporter 2 inhibitor, improves glycaemic control in subjects with type 2 diabetes inadequately controlled with diet and exercise

K. Stenlöf<sup>1</sup>, W.T. Cefalu<sup>2,3</sup>, C. Tong<sup>4</sup>, J. Yee<sup>4</sup>, S. Sha<sup>4</sup>, M. Alba<sup>4</sup>, W. Canovatchel<sup>4</sup>, G. Meininger<sup>4</sup>;<sup>1</sup>Clinical Trial Center, Sahlgrenska University Hospital, Gothenburg, Sweden,<sup>2</sup>Pennington Biomedical Research Center, Baton Rouge, USA, <sup>3</sup>LSUHSC School of Medicine, New Orleans, USA, <sup>4</sup>Janssen Research & Development, L.L.C., Raritan, USA.

**Background and aims:** Canagliflozin (CANA) is a novel inhibitor of the sodium glucose co-transporter 2 in development for treating patients with type 2 diabetes mellitus (T2DM). This study evaluated the efficacy and safety of CANA monotherapy in subjects with T2DM inadequately controlled with diet and exercise.

**Materials and methods:** In this randomised, double-blind, placebo (PBO)-controlled, Phase 3 study, subjects with T2DM (N = 584) received CANA 100 or 300 mg or PBO once daily. Changes from baseline in glycaemic and other efficacy parameters were assessed at 26 weeks. Adverse events (AEs) were recorded throughout the study.

**Results:** Mean baseline characteristics were similar across groups (age, 55.4 years; HbA<sub>1c</sub>, 8.0%; body weight, 86.8 kg; body mass index, 31.6 kg/m<sup>2</sup>); 48.1% of subjects were on background anti-hyperglycaemic agents. As shown in the Table, mean HbA<sub>1c</sub> was substantively and statistically significantly reduced from baseline at 26 weeks with CANA 100 and 300 mg compared with PBO ( $P < 0.001$  for both). Both CANA doses showed statistically significant improvements in fasting plasma glucose (FPG) and 2-hour postprandial glucose (PPG) compared with PBO ( $P < 0.001$  for all). More PBO-treated subjects received rescue therapy (22.9%) than those treated with CANA 100 or 300 mg (2.6% and 2.0%, respectively). In addition, both CANA doses significantly reduced body weight, decreased systolic blood pressure (BP), and increased high-density lipoprotein cholesterol (HDL-C) compared with PBO ( $P < 0.001$  for all), with a small dose-related increase in low-density lipoprotein cholesterol (LDL-C). Overall incidences of AEs were modestly higher with CANA 100 and 300 mg (60.5% and 59.9%, respectively) compared with PBO (49.0%). Rates of serious AEs (CANA 100 mg, 4.1%; CANA 300 mg, 1.0%; PBO, 2.1%) and AE-related discontinuations (CANA 100 mg, 3.1%; CANA 300 mg, 2.0%; PBO, 1.0%) were low across groups. Incidences of AEs consistent with superficial genital fungal infections were higher with CANA in women (CANA 100 mg, 12.3%; CANA 300 mg, 12.0%; PBO, 3.8%) and men (CANA 100 mg, 2.5%; CANA 300 mg, 5.6%; PBO, 0%). A slightly higher incidence of urinary tract infections was seen with CANA (CANA 100 mg, 7.2%; CANA 300 mg, 5.1%; PBO, 4.2%), as well as osmotic diuresis-related AEs (eg, polyuria;  $< 3\%$  per specific AE); these were generally mild and led to few discontinuations. No pyelonephritis AEs were reported in any group. Incidences of hypoglycaemia were similar across groups (CANA 100 mg, 3.1%; CANA 300 mg, 3.0%; PBO, 2.6%).

**Conclusion:** Treatment with CANA 100 and 300 mg significantly improved glycaemic control and reduced body weight, and was well tolerated in subjects with T2DM inadequately controlled with diet and exercise.



**Table. Summary of Efficacy Endpoints at Week 26 (LOCF)**

Parameter, mean (SE) <sup>a,b</sup>	CANA 100 mg (N = 195)	CANA 300 mg (N = 197)	PBO (N = 192)
HbA <sub>1c</sub> change, %	-0.77 (0.07)	-1.03 (0.06)	0.14 (0.07)
Difference vs PBO	-0.91 (0.09) <sup>c</sup>	-1.16 (0.09) <sup>c</sup>	
FPG change, mmol/L	-1.5 (0.1)	-1.9 (0.1)	0.5 (0.1)
Difference vs PBO	-2.0 (0.2) <sup>c</sup>	-2.4 (0.2) <sup>c</sup>	
2-hr PPG change, mmol/L	-2.4 (0.2)	-3.3 (0.2)	0.3 (0.2)
Difference vs PBO	-2.7 (0.3) <sup>c</sup>	-3.6 (0.3) <sup>c</sup>	
Body weight % change	-2.8 (0.2)	-3.9 (0.2)	-0.6 (0.2)
Difference vs PBO	-2.2 (0.3) <sup>c</sup>	-3.3 (0.3) <sup>c</sup>	
Systolic BP change, mmHg	-3.3 (0.8)	-5.0 (0.8)	0.4 (0.8)
Difference vs PBO	-3.7 (1.1) <sup>c</sup>	-5.4 (1.1) <sup>c</sup>	
Diastolic BP change, mmHg	-1.7 (0.5)	-2.1 (0.5)	-0.1 (0.5)
Difference vs PBO	-1.6 (0.7)	-2.0 (0.7)	
Triglycerides % change	2.5 (3.4)	-2.3 (3.4)	7.9 (3.5)
Difference vs PBO	-5.4 (4.8) <sup>c</sup>	-10.2 (4.8) <sup>c</sup>	
LDL-C % change	2.9 (1.8)	7.1 (1.8)	1.0 (1.9)
Difference vs PBO	2.0 (2.6)	6.1 (2.6)	
HDL-C % change	11.2 (1.4)	10.6 (1.4)	4.5 (1.4)
Difference vs PBO	6.8 (1.9) <sup>c</sup>	6.1 (1.9) <sup>c</sup>	
LDL-C/HDL-C % change	-5.8 (1.8)	-1.0 (1.8)	-1.9 (1.9)
Difference vs PBO	-4.0 (2.6)	0.9 (2.6)	

LOCF, last observation carried forward; SE, standard error; ANCOVA, analysis of covariance;

NS, not significant. <sup>a</sup>Least squares mean (SE) change from baseline using ANCOVA;<sup>b</sup>P values are reported for pre-specified comparisons only; <sup>c</sup>P < 0.001 vs PBO;<sup>d</sup>P = NS vs PBO; <sup>e</sup>P < 0.05 vs PBO; <sup>f</sup>P < 0.01 vs PBO.

Clinical Trial Registration Number: NCT01081834

Supported by: Janssen Research &amp; Development, L.L.C.

## 761

**Canagliflozin, a sodium glucose co-transporter 2 (SGLT2) inhibitor, improves indices of beta cell function in patients with type 2 diabetes on metformin plus sulphonylurea**

D. Polidori<sup>1</sup>, F. Vercruysse<sup>2</sup>, E. Ferrannini<sup>3</sup>;

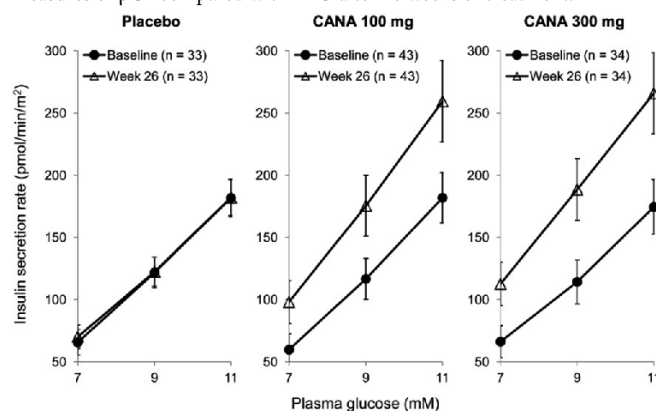
<sup>1</sup>Janssen Research & Development, L.L.C., San Diego, USA, <sup>2</sup>Janssen Research & Development, Beerse, Belgium, <sup>3</sup>University of Pisa School of Medicine, Italy.

**Background and aims:** Progressive loss of  $\beta$ -cell function ( $\beta$ CF) is thought to underlie the pathophysiology of type 2 diabetes mellitus (T2DM). Canagliflozin (CANA), an inhibitor of SGLT2, is in development for the treatment of T2DM. In hyperglycaemic, partially pancreatectomised rats,  $\beta$ CF can be restored when normoglycaemia is achieved with SGLT2 inhibitor treatment. In a Phase 3 study, 26 weeks of CANA monotherapy treatment improved glycaemic control and indices of  $\beta$ CF in patients with T2DM inadequately controlled with diet and exercise. To further assess the effects of CANA on  $\beta$ CF, indices of  $\beta$ CF were evaluated in a Phase 3 study of patients with T2DM inadequately controlled with metformin (MET) + sulphonylurea (SU).

**Materials and methods:** This 26-week, randomised, double-blind, placebo (PBO)-controlled study evaluated CANA 100 and 300 mg compared with PBO as add-on therapy to MET + SU in patients with T2DM (N = 469; mean baseline characteristics: age = 57 years; HbA<sub>1c</sub> = 8.1%; BMI = 33.0 kg/m<sup>2</sup>; duration of T2DM = 9.6 years). A subset of patients (n = 168) were given a meal tolerance test at baseline and Week 26 and plasma glucose (G) and serum C-peptide (C) were measured at 7 time points over 3 hours.  $\beta$ CF was assessed using the AUC<sub>C</sub>/AUC<sub>G</sub> ratio and a model-based method relating the insulin secretion rate (ISR; obtained by deconvolution of C) to concomitant G using linear regression.  $\beta$ -cell glucose sensitivity as the slope of ISR vs G relationship and ISR at 9 mM G were calculated. All changes are reported as PBO-subtracted least squares (LS) mean changes using an ANCOVA model.

**Results:** At Week 26, CANA 100 and 300 mg significantly reduced HbA<sub>1c</sub> from baseline compared with PBO ( $\Delta$ HbA<sub>1c</sub> = -0.71% and -0.92%, respectively;  $P < 0.001$ ) and were generally well tolerated. The ISR vs G relationship was unchanged with PBO and was shifted upwards (indicating increased ISR at each PG concentration) with both CANA doses (Figure). Mean values of all calculated indices of  $\beta$ CF increased with both CANA doses versus PBO, although some of the changes did not reach statistical significance. Mean AUC<sub>C</sub>/AUC<sub>G</sub> increased by ~20% from baseline values of 123–132 pM/mM (increases of 27.1 (95% CI = -0.2; 54.4;  $P = 0.051$ ) and 27.4 (-0.7; 55.6;  $P = 0.056$ ) for CANA 100 and 300 mg). Mean ISR at 9 mM G increased by ~50–60% from baseline values of 114–117 pmol/min/m<sup>2</sup> (increases of 54.6 (7.7; 101.5;  $P = 0.02$ ) and 69.2 (19.7; 118.9;  $P = 0.007$ ) for CANA 100 and 300 mg). Mean  $\beta$ -cell glucose sensitivity increased by ~20% from baseline values of 31–34 pmol·min<sup>-1</sup>·m<sup>-2</sup>·mM<sup>-1</sup> (increases of 7.6 (-2.6; 17.9;  $P = 0.14$ ) and 6.7 (-4.1; 17.5;  $P = 0.22$ ) for CANA 100 and 300 mg).

**Conclusion:** In T2DM patients (mean T2DM duration of 9.6 years) on background MET + SU, CANA 100 and 300 mg improved glycaemic control and measures of  $\beta$ CF compared with PBO after 26 weeks of treatment.

Values shown are mean  $\pm$  SEM for subjects studied at both baseline and Week 26

Clinical Trial Registration Number: NCT01106625

Supported by: Janssen Research &amp; Development, L.L.C.

## 762

**Canagliflozin, a sodium glucose co-transporter 2 inhibitor, reduces body weight mainly through loss of fat mass in subjects with type 2 diabetes**

S. Toubro<sup>1</sup>, W.T. Cefalu<sup>2,3</sup>, J. Xie<sup>4</sup>, D. Sullivan<sup>4</sup>, K. Usiskin<sup>4</sup>, W. Canovatchel<sup>4</sup>, G. Meininger<sup>4</sup>;

<sup>1</sup>Reduce, A Research Clinic, Vipperød, Denmark, <sup>2</sup>Pennington Biomedical Research Center, Baton Rouge, USA, <sup>3</sup>LSUHSC School of Medicine, New Orleans, USA, <sup>4</sup>Janssen Research & Development, L.L.C., Raritan, USA.

**Background and aims:** Canagliflozin (CANA) is a sodium glucose co-transporter 2 inhibitor that improves the glycaemic profile and reduces body weight in type 2 diabetes mellitus (T2DM) subjects. Body composition analysis was conducted to determine the relative contributions of weight loss from fat and lean mass in a subset of subjects with T2DM enrolled in 2 randomised, double-blind, Phase 3 studies.

**Materials and methods:** Study 1 was an active-controlled trial comparing CANA 100 and 300 mg with glimepiride (GLIM) in T2DM subjects inadequately controlled on metformin (N = 1,450; mean baseline characteristics: age, 56.2 years; HbA<sub>1c</sub>, 7.8%; body weight, 86.6 kg). Study 2 was a placebo (PBO)-controlled trial that evaluated CANA 100 and 300 mg in subjects aged 55 to 80 years (N = 714) with T2DM on a variety of background anti-hyperglycaemic agents (AHAs); mean baseline characteristics were similar across groups (age, 63.6 years; HbA<sub>1c</sub>, 7.7%; body weight, 89.5 kg). Body composition was assessed in a subset of subjects by dual-energy X-ray absorptiometry (DXA) at 52 weeks in Study 1 (n = 208) and at 26 weeks in Study 2 (n = 211). Abdominal fat distribution was also assessed by computed tomography (CT) scans at 52 weeks in Study 1 only (n = 217).

**Results:** In T2DM subjects on background metformin (Study 1 [Table]), CANA 100 and 300 mg significantly reduced body weight compared with GLIM ( $P < 0.001$  for both) at 52 weeks (least squares [LS] mean changes from baseline of -4.2, -4.7, and 1.0 kg, respectively). Loss of fat mass accounted for approximately two-thirds of the overall body weight reduction seen with CANA. Both CANA doses also showed reductions in percent total fat (fat percentage of the total weight) compared with GLIM. Similar results were observed in Study 2 subjects on a variety of background AHAs (Table). CANA 100 and 300 mg significantly reduced body weight compared with PBO ( $P < 0.001$  for both) after 26 weeks of treatment (LS mean changes from baseline of -2.5, -3.3, and -0.2 kg, respectively). Greater loss from fat mass than from lean mass was observed for CANA 100 and 300 mg, and both CANA doses showed reductions compared with PBO in percent total fat. Analysis of abdominal fat in Study 1 showed slightly greater changes in the amount of visceral adipose tissue (LS mean changes relative to GLIM of -7.4% and -8.3%, respectively) than changes in subcutaneous adipose tissue (LS mean changes relative to GLIM of -7.2% and -7.4%, respectively) with CANA 100 and 300 mg.

**Conclusion:** The body weight reductions seen in subjects with T2DM following 26 or 52 weeks of treatment with CANA 100 or 300 mg were predominantly (>2/3) from fat mass. There was a numerically greater percentage loss of visceral relative to subcutaneous fat in the abdomen.

Table. Summary of Body Composition Endpoints

Study 1, Week 52 (LOCF)			
Parameter <sup>a</sup>	CANA 100 mg (N = 71)	CANA 300 mg (N = 69)	GLIM (N = 68)
Body weight % change	-5.0 (0.7)	-4.9 (0.7)	1.4 (0.8)
Difference vs GLIM	-6.4 (-7.5, -5.2)	-6.2 (-7.4, -5.1)	
DXA			
Total fat measurement change, kg	-2.9 (0.5)	-2.5 (0.5)	1.0 (0.5)
Difference vs GLIM	-3.9 (-4.8, -3.1)	-3.5 (-4.4, -2.6)	
Total lean measurement change, kg	-0.9 (0.3)	-1.1 (0.3)	1.1 (0.3)
Difference vs GLIM	-1.9 (-2.5, -1.3)	-2.2 (-2.8, -1.6)	
Percent total fat change, % <sup>b</sup>	-2.0 (0.4)	-1.5 (0.5)	0.7 (0.4)
Difference vs GLIM	-2.6 (-3.3, -1.9)	-2.2 (-3.0, -1.4)	
Study 2, Week 26 (LOCF)			
Parameter <sup>a</sup>	CANA 100 mg (N = 63)	CANA 300 mg (N = 73)	PBO (N = 75)
Body weight % change	-2.5 (0.4)	-3.3 (0.4)	-0.2 (0.4)
Difference vs PBO	-2.3 (-3.3, -1.4)	-3.1 (-4.0, -2.2)	
DXA			
Total fat measurement change, kg	-1.8 (0.3)	-2.5 (0.3)	-0.3 (0.3)
Difference vs PBO	-1.5 (-2.3, -0.7)	-2.2 (-2.9, -1.4)	
Total lean measurement change, kg	-1.1 (0.3)	-1.1 (0.3)	-0.3 (0.3)
Difference vs PBO	-0.7 (-1.4, -0.1)	-0.8 (-1.4, -0.2)	
Percent total fat change, % <sup>b</sup>	-0.9 (0.3)	-1.3 (0.3)	-0.02 (0.3)
Difference vs PBO	-0.9 (-1.5, -0.3)	-1.3 (-1.9, -0.7)	

LOCF, last observation carried forward; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. <sup>a</sup>Least squares mean (SE) change from baseline using ANCOVA and difference (95% CI) vs GLIM (Study 1) or PBO (Study 2). <sup>b</sup>Percent total fat (%) = Total fat measurement (kg)/(Total fat measurement [kg] + Total lean measurement [kg] + bone mineral content [kg]).

Clinical Trial Registration Number: NCT00968812 & NCT01106651  
Supported by: Janssen Research & Development, L.L.C.

## 763

### Efficacy and safety of canagliflozin, a sodium glucose co-transporter 2 inhibitor, compared with glimepiride in patients with type 2 diabetes on background metformin

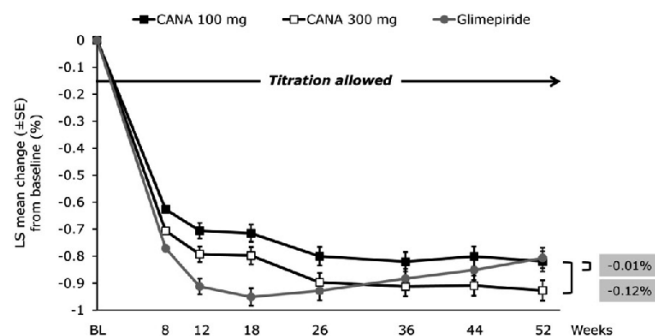
L. Niskanen<sup>1,2</sup>, W.T. Cefalu<sup>3,4</sup>, L.A. Leiter<sup>5</sup>, J. Xie<sup>6</sup>, D. Balis<sup>6</sup>, W. Canovatchel<sup>6</sup>, G. Meininger<sup>6</sup>

<sup>1</sup>Central Hospital Central Finland, Jyväskylä, Finland, <sup>2</sup>University of Eastern Finland, Kuopio, Finland, <sup>3</sup>Pennington Biomedical Research Center, Baton Rouge, USA, <sup>4</sup>LSUHSC School of Medicine, New Orleans, USA, <sup>5</sup>St. Michael's Hospital and University of Toronto, Canada, <sup>6</sup>Janssen Research & Development, L.L.C., Raritan, USA.

**Background and aims:** This 52-week study evaluated the efficacy and safety of canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor, compared with glimepiride (GLIM) in subjects with type 2 diabetes mellitus (T2DM) inadequately controlled with metformin (MET).

**Materials and methods:** In this large, randomised, double-blind, active-controlled, Phase 3 study (N = 1,450), subjects with T2DM inadequately controlled with MET received CANA 100 or 300 mg or GLIM (doses titrated over 52 weeks up to 6 or 8 mg/day, with mean dose achieved of 5.6 mg). Changes from baseline in efficacy endpoints were assessed at Week 52. Adverse events (AEs) were recorded throughout the study.

**Results:** Mean baseline characteristics were similar across groups (age, 56.2 years; HbA<sub>1c</sub>, 7.8%; body weight, 86.6 kg; body mass index, 31.0 kg/m<sup>2</sup>). As shown in the Figure, HbA<sub>1c</sub> (primary endpoint) was reduced from baseline at 52 weeks with CANA 100 and 300 mg and GLIM (least squares [LS] mean changes of -0.82%, -0.93%, and -0.81%). Both CANA doses demonstrated non-inferiority to GLIM in reducing HbA<sub>1c</sub>, and CANA 300 mg demonstrated superiority to GLIM. CANA 100 and 300 mg provided consistently lower HbA<sub>1c</sub> over 52 weeks while GLIM showed increases in HbA<sub>1c</sub> after Week 18. CANA 100 and 300 mg were superior to GLIM (*P* < 0.001 for all) in body weight reduction (LS mean changes of -4.2, -4.7, and 1.0 kg) and documented hypoglycaemia (defined as ≤3.9 mmol/L) rates (5.6%, 4.9%, and 34.2%). Both CANA doses showed improvements in fasting plasma glucose (FPG), systolic blood pressure (BP), triglycerides, and high-density lipoprotein cholesterol (HDL-C); there was a small dose-related increase in low-density lipoprotein cholesterol (LDL-C) with CANA. More GLIM-treated subjects received rescue therapy (10.6%) than with CANA 100 mg (6.6%) or 300 mg (4.9%).



The overall incidence of AEs was similar with CANA 100 mg (64.2%), CANA 300 mg (68.5%), and GLIM (67.6%). Rates of serious AEs (CANA 100 mg, 5.0%; CANA 300 mg, 4.9%; GLIM, 7.9%) and AE-related discontinuations (CANA 100 mg, 5.2%; CANA 300 mg, 6.8%; GLIM, 5.8%) were low and similar across groups. AEs consistent with superficial genital fungal infections were more frequent with CANA 100 and 300 mg than with GLIM in women (14.3% and 23.8% vs 3.7%) and men (6.7% and 8.3% vs 1.1%). CANA showed slightly higher rates relative to GLIM of urinary tract infections (6.4% for both doses vs 4.4%) and osmotic diuresis-related AEs (eg, pollakiuria; <3% for each specific AE).

**Conclusion:** CANA showed consistent HbA<sub>1c</sub> lowering over 52 weeks and reduced body weight compared with GLIM, and was well tolerated in subjects with T2DM inadequately controlled with MET.

Clinical Trial Registration Number: NCT00968812

Supported by: Janssen Research & Development, L.L.C.

## 764

### Efficacy and safety of canagliflozin (CANA), an inhibitor of sodium glucose co-transporter 2 (SGLT2), added-on to insulin therapy +/- oral agents in type 2 diabetes

D.R. Matthews<sup>1</sup>, G. Fulcher<sup>2</sup>, V. Perkovic<sup>3</sup>, D. de Zeeuw<sup>4</sup>, K.W. Mahaffey<sup>5</sup>, J. Rosenstock<sup>6</sup>, M. Davies<sup>7</sup>, G. Capuano<sup>8</sup>, M. Desai<sup>8</sup>, W. Shaw<sup>8</sup>, F. Vercruysee<sup>8</sup>, G. Meininger<sup>8</sup>, B. Neal<sup>3</sup>

<sup>1</sup>The Churchill Hospital, Oxford, UK, <sup>2</sup>Royal North Shore Hospital, Sydney, Australia, <sup>3</sup>George Inst for Global Health, Sydney, Australia, University Med Ctr Groningen, Netherlands, <sup>4</sup>Duke Clin Res Inst, Durham, USA, <sup>5</sup>Dallas Diab & Endo Ctr, Dallas, USA, University of Leicester, UK, <sup>8</sup>Janssen R&D, L.L.C., Raritan, USA.

**Background and aims:** Inhibition of SGLT2 is a novel modality of treatment for patients with T2D. We report here a pre-specified substudy of the canagliflozin Cardiovascular Assessment Study (CANVAS) defining the efficacy, safety and tolerability of CANA in participants with T2D on insulin.

**Materials and methods:** CANVAS is a double-blind, placebo (PBO)-controlled study that randomised 4,330 individuals at elevated cardiovascular risk to the addition of PBO, CANA 100 or 300 mg, once daily, to stable ongoing diabetes therapy. In the subgroup using insulin (≥30 units/d), there were 565, 566 and 587 individuals, respectively. The primary endpoint for this sub-study was the change from baseline in HbA<sub>1c</sub> at 18 weeks follow-up while the insulin doses were kept unchanged as per protocol. Major vascular outcomes remained blinded.

**Results:** Baseline demographics demonstrated no imbalances; 67% of participants were male and 59% had a history of a prior vascular event. Mean baseline age was 63 y, HbA<sub>1c</sub> 8.3%, BMI 33.8 kg/m<sup>2</sup>, body weight 97.0 kg, fasting plasma glucose 9.4 mmol/L, total cholesterol 4.3 mmol/L and blood pressure 138/76 mmHg. Mean daily insulin dose at baseline was 83 IU with most using basal/bolus insulin regimens and a median duration of diabetes of 15 y. Compared to PBO both doses of CANA significantly improved measures of glycaemia (Table). Adverse events (AEs) were reported for the CANA 100 mg, CANA 300 mg and PBO groups in 63%, 65% and 59% of participants and serious AEs in 5.5%, 4.9% and 6.4% respectively. Incidence of AEs leading to discontinuation was greater with CANA 300 mg (5.3%) compared to CANA 100 mg or PBO (both 1.9%). Male and female genital fungal infections were more common with CANA 100 mg (F:11.8%, M:4.0%) and 300 mg (F:9.9%, M:8.3%) compared to PBO (F:2.2%, M:0.5%) as were AEs of pollakiuria and hypotension with CANA 100 mg (3.7% and 1.2%, respectively) and CANA 300 mg (5.6% and 2.6%, respectively), compared to PBO (0.5% and 0%, respectively). A slightly higher incidence of UTI was seen with CANA 300 mg (3.4%) relative to CANA 100 mg and PBO (2.3% and 2.1%, respectively). In-



cidence of hypoglycaemia was higher with CANA 100 mg (49%) and CANA 300 mg (48%) than PBO (37%).

**Conclusion:** CANA added to stable insulin therapy improved glycaemic control and produced significant improvements in a number of efficacy parameters important in the management of T2D with effects of the 300 mg dose numerically greater than the 100 mg dose. CANA was generally well tolerated, and associated with a greater frequency of genital fungal infections and slightly higher risk of hypoglycaemia.

Table. Effects on primary and secondary outcomes\*

	Difference CANA 100 mg vs. placebo	Difference CANA 300 mg vs. placebo
HbA <sub>1c</sub> %	-0.65 (-0.73 to -0.56), p<0.001	-0.73 (-0.81 to -0.65), p<0.001
% change in body weight	-1.9 (-2.2 to -1.6), p<0.001	-2.4 (-2.7 to -2.1), p<0.001
FPG (mmol/L)	-1.25 (-1.55 to -0.96), p<0.001	-1.61 (-1.90 to -1.31), p<0.001
Proportion (%) HbA <sub>1c</sub> <7%	12.1 (7.8 to 16.3), p<0.001	17.0 (12.6 to 21.4), p<0.001
SBP (mmHg)	-2.6 (-4.1 to -1.1), p<0.001	-4.4 (-5.9 to -2.9), p<0.001
DBP (mmHg)	-1.0 (-1.9 to -0.1), p=0.04	-1.8 (-2.7 to -1.0), p=0.001
% change in HDL-C	0.8 (-1.4 to 3.0), p=0.46	4.7 (2.5 to 6.8), p<0.001
% change in Triglycerides	0.2 (-4.9 to 5.2), p=0.95	-2.0 (-7.0 to 3.0), p=0.44
% change in LDL-cholesterol	6.3 (-1.2 to 13.9), p=0.09	6.6 (-0.9 to 14.1), p=0.08
% change in Total cholesterol	1.0 (-1.2 to 3.1), p=0.38	3.3 (1.2 to 5.4), p=0.001
% change in LDL:HDL cholesterol	4.9 (-2.7 to 12.5), p=0.19	1.9 (-5.6 to 9.4), p=0.78

Most effect estimates and p-values calculated using analysis of covariance; logistic regression was used for proportion (%) HbA<sub>1c</sub> <7%

\*P-values provided for endpoints that were pre-specified for hypothesis testing

Clinical Trial Registration Number: NCT01032629

Supported by: Janssen Research & Development, L.L.C.

## 765

### Efficacy and safety of canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor (SGLT2), in older subjects with type 2 diabetes mellitus

B. Bode<sup>1</sup>, K. Stenlöf<sup>2</sup>, D. Sullivan<sup>3</sup>, A. Fung<sup>3</sup>, K. Usiskin<sup>3</sup>, G. Meininger<sup>3</sup>;

<sup>1</sup>Atlanta Diabetes Associates, Atlanta, USA, <sup>2</sup>Clinical Trial Center,

Sahlgrenska University Hospital, Gothenburg, Sweden, <sup>3</sup>Janssen Research & Development, L.L.C., Raritan, USA.

**Background and aims:** The efficacy and safety of CANA, an inhibitor of the SGLT2, were evaluated in this randomised, double-blind, placebo (PBO)-controlled, Phase 3 study in older subjects with T2DM inadequately controlled with anti-hyperglycaemic agents (AHAs).

**Materials and methods:** Subjects aged 55 to 80 years with T2DM (N = 714) inadequately controlled with a stable AHA regimen received CANA 100 or 300 mg or PBO daily. Efficacy endpoints were evaluated at 26 weeks and safety was assessed by adverse event (AE) reports. Changes in bone biomarkers and bone density (measured by DXA at 4 sites: lumbar spine, total hip, femoral neck, distal forearm) were also determined.

**Results:** Mean baseline characteristics were similar across groups (age, 63.6 years; HbA<sub>1c</sub>, 7.7%; body weight, 89.5 kg). Background AHAs included metformin (85.3%), sulphonylureas (48.7%), and insulin (32.7%), with 76% of subjects on ≥2 classes of AHAs at baseline. As shown in the Table, CANA 100 and 300 mg showed significant reductions in HbA<sub>1c</sub> and improvements in fasting plasma glucose at Week 26 compared with PBO (P <0.001 for all). Both CANA doses significantly reduced body weight and improved systolic blood pressure (BP) and high-density lipoprotein cholesterol (HDL-C) (P <0.001 for all), with small increases observed in low-density lipoprotein cholesterol (LDL-C) compared with PBO.

Table. Summary of Efficacy Endpoints at Week 26 (LOCF)

Parameter, mean (SE) <sup>a,b</sup>	CANA 100 mg (N = 241)	CANA 300 mg (N = 236)	PBO (N = 237)
HbA <sub>1c</sub> change, %	-0.60 (0.06)	-0.73 (0.06)	-0.03 (0.06)
Difference vs PBO	-0.57 (0.07) <sup>c</sup>	-0.70 (0.07) <sup>c</sup>	
FPG change, mmol/L	-1.0 (0.2)	-1.1 (0.2)	0.4 (0.2)
Difference vs PBO	-1.4 (0.2) <sup>c</sup>	-1.5 (0.2) <sup>c</sup>	
Body weight % change	-2.4 (0.3)	-3.1 (0.3)	-0.1 (0.3)
Difference vs PBO	-2.3 (0.3) <sup>c</sup>	-3.0 (0.3) <sup>c</sup>	
Systolic BP change, mmHg	-3.5 (1.0)	-6.8 (1.1)	1.1 (1.0)
Difference vs PBO	-4.6 (1.1) <sup>c</sup>	-7.9 (1.1) <sup>c</sup>	
Diastolic BP change, mmHg	-1.6 (0.6)	-3.2 (0.6)	0.1 (0.6)
Difference vs PBO	-1.6 (0.6)	-3.2 (0.7)	
Triglycerides % change	2.8 (3.3)	8.4 (3.4)	7.6 (3.4)
Difference vs PBO	-4.8 (3.7) <sup>d</sup>	0.7 (3.7) <sup>d</sup>	
LDL-C % change	14.2 (3.2)	14.5 (3.3)	6.7 (3.3)
Difference vs PBO	7.5 (3.6)	7.8 (3.6)	
HDL-C % change	6.8 (1.2)	6.2 (1.2)	1.5 (1.2)
Difference vs PBO	5.3 (1.4) <sup>c</sup>	4.7 (1.4) <sup>c</sup>	
LDL-C/HDL-C % change	8.7 (3.4)	11.5 (3.4)	5.6 (3.5)
Difference vs PBO	3.1 (3.8)	5.9 (3.8)	

LOCF, last observation carried forward; SE, standard error; ANCOVA, analysis of covariance; NS, not significant. <sup>a</sup>Least squares mean (SE) change from baseline using ANCOVA; <sup>b</sup>P values are reported for pre-specified comparisons only; <sup>c</sup>P <0.001 vs PBO; <sup>d</sup>P = NS vs PBO.

The overall incidence of AEs was similar with CANA 100 and 300 mg and PBO (71.8% and 78.0% vs 73.4%). Rates of serious AEs (4.1% and 3.4% vs 5.1%) and AE-related discontinuations (2.1% and 7.2% vs 4.2%) were low across groups. CANA 100 and 300 mg showed higher rates than PBO of superficial genital fungal infections (women, 18.8% and 17.8% vs 4.3%; men, 3.2% and 6.2% vs 0%), urinary tract infections (5.8% and 8.1% vs 5.1%), and osmotic diuresis-related AEs (<6% per specific AE). Hypoglycaemia (≤3.9 mmol/L) rates were modestly higher with CANA 100 and 300 mg than PBO in subjects on an AHA associated with hypoglycaemia (43.1% and 47.4% vs 37.7%) as well as those on AHAs not associated with hypoglycaemia (6.7% and 4.8% vs 3.2%). CANA 100 and 300 mg showed small increases (16.2% and 23.9% [PBO-subtracted]) in serum collagen type 1 beta-carboxy telopeptide (beta-CTX), a bone resorption marker, and small decreases (-5.5% and -6.8% [PBO-subtracted]) in serum propeptide amino-term type 1 procollagen (PINP), a bone formation marker. There were no discernable changes in bone density seen at the spine, hip, or distal forearm.

**Conclusion:** CANA 100 and 300 mg improved glycaemic control, reduced body weight and systolic BP, and was well tolerated in older subjects with T2DM inadequately controlled with a variety of background AHAs, with no discernable changes in cortical or trabecular bone density.

Clinical Trial Registration Number: NCT01106651

Supported by: Janssen Research & Development, L.L.C.

## 766

### Canagliflozin, a sodium glucose co-transporter 2 inhibitor, improves glycaemia in subjects with type 2 diabetes inadequately controlled with metformin plus sulphonylurea

J. Wilding<sup>1</sup>, C. Mathieu<sup>2</sup>, L. Deng<sup>3</sup>, S. Black<sup>3</sup>, F. Vercruysse<sup>4</sup>, W. Canovatchel<sup>3</sup>, G. Meininger<sup>3</sup>;

<sup>1</sup>University of Liverpool, Liverpool, UK, <sup>2</sup>KULeuven, Belgium, <sup>3</sup>Janssen

Research & Development, L.L.C., Raritan, USA, <sup>4</sup>Janssen Research & Development, Beerse, Belgium.

**Background and aims:** Canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor, is in development as a novel treatment for patients with type 2 diabetes mellitus (T2DM). This study assessed the efficacy and safety of CANA in subjects with T2DM who have inadequate glycaemic control with metformin (MET) plus sulphonylurea (SU) therapy.

**Materials and methods:** In this randomised, double-blind, placebo (PBO)-controlled, Phase 3 study, subjects with T2DM on MET + SU (N = 469) received daily treatment with CANA 100 mg or 300 mg or PBO. Changes from baseline in glycaemic and other efficacy parameters were evaluated at Week 26. Adverse events (AEs) were recorded throughout the study.

**Results:** Mean baseline characteristics were similar across groups (age, 56.7 years; HbA<sub>1c</sub>, 8.1%; body weight, 92.8 kg; body mass index, 33.0 kg/m<sup>2</sup>). As shown in the Table, HbA<sub>1c</sub> was significantly reduced from baseline at 26 weeks with CANA 100 and 300 mg compared with PBO (P <0.001 for both). Both CANA doses significantly improved fasting plasma glucose (FPG) compared with PBO (P <0.001 for both). More PBO-treated subjects received rescue therapy (12.8%) than those treated with CANA 100 or 300 mg (1.3% and 1.9%, respectively). Both CANA doses significantly reduced body weight compared with PBO (P <0.001 for both) and showed non-dose-dependent trends toward improvement in systolic blood pressure (BP), high-density lipoprotein cholesterol (HDL-C), and triglycerides (not statistically significant); there was a small increase seen in low-density lipoprotein cholesterol (LDL-C) with CANA 300 mg compared with PBO. Overall, the incidence of AEs was similar across groups (CANA 100 mg, 57.3%; CANA 300 mg, 62.2%; PBO, 63.5%). Rates of serious AEs (CANA 100 mg, 3.2%; CANA 300 mg, 3.8%; PBO, 5.8%) and AE-related discontinuations (CANA 100 mg, 5.7%; CANA 300 mg, 5.8%; PBO, 3.2%) were low. CANA showed higher rates of AEs consistent with superficial genital fungal infections in women (CANA 100 mg, 16.0%; CANA 300 mg, 21.7%; PBO, 5.0%) and men (CANA 100 mg, 6.6%; CANA 300 mg, 3.4%; PBO, 1.3%). Incidences of osmotic diuresis-related AEs were low, although numerically higher with CANA (<3% per specific AE), but none led to study discontinuation. Rates of urinary tract infections were similar among groups (CANA 100 mg, 6.4%; CANA 300 mg, 5.8%; PBO, 5.1%). More subjects treated with CANA had ≥1 hypoglycaemia episode (CANA 100 mg, 26.8%; CANA 300 mg, 30.1%; PBO, 15.4%), but the number of severe hypoglycaemia episodes was low (≤1 per group) and similar across groups.

**Conclusion:** Treatment with CANA 100 and 300 mg provided glycaemic improvement, reduced body weight, and was well tolerated in subjects with T2DM inadequately controlled with MET + SU.



**Table. Summary of Efficacy Endpoints at Week 26 (LOCF)**

Parameter, mean (SE) <sup>a,b</sup>	CANA 100 mg (N = 157)	CANA 300 mg (N = 156)	PBO (N = 156)
HbA <sub>1c</sub> change, %	−0.85 (0.08)	−1.06 (0.08)	−0.13 (0.08)
Difference vs PBO	−0.71 (0.10) <sup>c</sup>	−0.92 (0.10) <sup>c</sup>	
FPG change, mmol/L	−1.0 (0.2)	−1.7 (0.2)	0.2 (0.2)
Difference vs PBO	−1.2 (0.3) <sup>c</sup>	−1.9 (0.3) <sup>c</sup>	
Body weight % change	−2.1 (0.3)	−2.6 (0.3)	−0.7 (0.3)
Difference vs PBO	−1.4 (0.4) <sup>c</sup>	−2.0 (0.4) <sup>c</sup>	
Systolic BP change, mmHg	−4.9 (1.0)	−4.3 (1.0)	−2.7 (1.0)
Difference vs PBO	−2.2 (1.3) <sup>d</sup>	−1.6 (1.3) <sup>d</sup>	
Diastolic BP change, mmHg	−2.9 (0.6)	−2.3 (0.6)	−1.7 (0.6)
Difference vs PBO	−1.1 (0.8)	−0.5 (0.8)	
Triglycerides % change	5.4 (4.2)	8.5 (4.2)	11.6 (4.2)
Difference vs PBO	−6.2 (5.4) <sup>d</sup>	−3.1 (5.5) <sup>d</sup>	
LDL-C % change	3.8 (2.5)	7.8 (2.5)	3.3 (2.5)
Difference vs PBO	0.5 (3.2)	4.6 (3.2)	
HDL-C % change	5.7 (1.3)	6.6 (1.3)	3.1 (1.3)
Difference vs PBO	2.6 (1.7) <sup>d</sup>	3.5 (1.7) <sup>d</sup>	
LDL-C/HDL-C % change	−0.8 (2.5)	2.2 (2.5)	1.9 (2.5)
Difference vs PBO	−2.7 (3.2)	0.3 (3.2)	

LOCF, last observation carried forward; SE, standard error; ANCOVA, analysis of covariance; NS, not significant. <sup>a</sup>Least squares mean (SE) change from baseline using ANCOVA. <sup>b</sup>P values are reported for pre-specified comparisons only; <sup>c</sup>P < 0.001 vs PBO; <sup>d</sup>P = NS vs PBO.

Clinical Trial Registration Number: NCT01106625

Supported by: Janssen Research & Development, L.L.C.

## 767

**Tofogliflozin a selective SGLT2 inhibitor exhibits highly favourable drug properties for use in patients with renal impairment and for combination with other medicines**

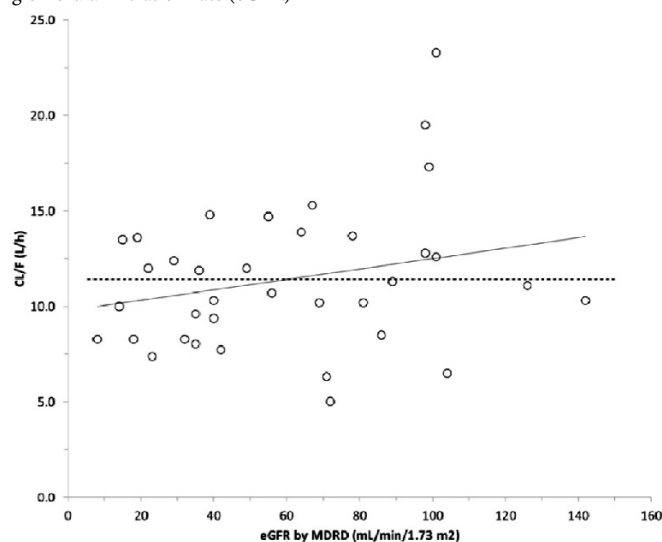
D. Schwab<sup>1</sup>, A. Portron<sup>1</sup>, Y. Fukushima<sup>1</sup>, Z. Backholer<sup>1</sup>, O. Kuhlmann<sup>1</sup>, T. Saito<sup>2</sup>, S. Ikeda<sup>2</sup>, Hoffmann-La Roche AG, Basel, Switzerland, <sup>2</sup>Chugai Pharmaceutical Co., Ltd, Tokyo, Japan.

**Background and aims:** Tofogliflozin (TOFO) is a novel selective SGLT2 inhibitor increasing glucosuria by inhibition of glucose reabsorption in the kidney for the treatment of type 2 diabetes mellitus (T2DM). Patients with T2DM exhibit various degrees of renal impairment and take a variety of co-medications. Therefore, we investigated the suitability of TOFO to be administered to patients with impaired renal function as well as the potential of the drug-drug interactions of TOFO and common co-medications.

**Materials and methods:** TOFO was characterized in several clinical pharmacology studies following single dose administration: 1) concomitant oral and intravenous microdose administration as part of an ADME investigation in healthy subjects, 2) drug-drug interaction study in healthy subjects, 3) in patients with T2DM with varying degree of renal function. In vitro data was obtained in human liver microsomes.

**Results:** Renal clearance of TOFO accounts for 19% of total systemic clearance. A study in 36 T2DM patients with varying degree of renal function (eGFR from 8 to >90 mL/min) demonstrated that impaired renal function does not affect systemic exposure of TOFO significantly (figure). Although lower urinary glucose excretion correlated with lower eGFR, the inhibition of renal glucose reabsorption was constant across all renal function groups. This indicates that TOFO reached its target in the kidney independent on renal function. Several preclinical and clinical studies support TOFO's low risk for drug-drug interactions with concomitant use of medications in respect to both TOFO as a victim and as a perpetrator of drug-drug interactions. In vitro data shows lack of a relevant interference of TOFO with CYP enzymes and drug transporters suggesting that TOFO is unlikely to cause a relevant change in exposures of co-medications. The susceptibility of TOFO to become a victim of a drug-drug interaction was assessed by combining in vitro data and clinical data from a mass balance study. TOFO is eliminated via multiple elimination pathways, suggesting minor susceptibility for drug-drug interactions. Moreover, the main metabolic pathway is predominantly driven by alcohol dehydrogenase and additional enzymes. The multiplicity of enzymes involved in the main metabolic pathway, and supportive clinical data suggest a low potential for drug-drug interactions.

**Conclusion:** TOFO exhibits highly favorable clinical and in vitro drug properties for the use in the target population that is subject to a variety of co-medications and which presents with various degrees of renal function.

**Figure:** Relationship between oral clearance (CL/F) of TOFO and estimated glomerular filtration rate (eGFR)

Clinical Trial Registration Number: NCT00933972

Supported by: Roche and Chugai

## 768

**A novel and selective SGLT2 inhibitor, tofogliflozin improves glycaemic control and lowers body weight in patients with type 2 diabetes mellitus**  
S. Ikeda<sup>1</sup>, Y. Takano<sup>1</sup>, O. Cynshi<sup>1</sup>, A.D. Christ<sup>2</sup>, V. Boerlin<sup>2</sup>, U. Beyer<sup>2</sup>, A. Beck<sup>2</sup>, <sup>1</sup>Chugai Pharmaceutical Co., Ltd, Tokyo, Japan, <sup>2</sup>F. Hoffmann-La Roche AG, Basel, Switzerland.

**Background and aims:** Sodium-glucose cotransporter 2 (SGLT2) inhibitor is expected to be an emerging oral antidiabetic with insulin independent mode of action. Urinary glucose excretion (UGE) induced by selective inhibition of SGLT2 elicits a complex set of metabolic changes on top of blood glucose lowering and ameliorates type 2 diabetes mellitus (T2DM). Tofogliflozin is a novel and highly selective SGLT2 inhibitor.

**Materials and methods:** Safety, tolerability and efficacy of tofogliflozin was evaluated in a double-blind, randomized, placebo-controlled 12-week dose finding study. After 4-week placebo run-in period, total 398 T2DM patients were randomized to tofogliflozin 2.5, 5, 10, 20, 40 mg or placebo (qd, 15 min before breakfast). Treatment background in the study consisted of (1) treated with diet and exercise and a stable dose of metformin (approx.60% of total) or (2) treated with diet and exercise alone (remaining 40%).

**Results:** Significant (except for 2.5 mg) and dose-dependent reductions of HbA<sub>1c</sub> were shown with a maximum lowering of 0.56% (placebo-adjusted, at 40 mg), along with increased UGE. No clear difference was found between two treatment backgrounds. Dose-dependent fasting plasma glucose and body weight reduction were also observed (Table), glucose intolerance was improved and there was a trend for median blood pressure to decrease by 3-4 mmHg.

**Conclusion:** Once daily doses of tofogliflozin for 12 weeks were effective, well tolerated and no new clinical safety concerns were identified in addition to the known genital infections reported with other SGLT2 inhibitors in development.

Table	2.5 mg (n=65)	5 mg (n=64)	10 mg (n=65)	20 mg (n=66)	40 mg (n=64)	40 mg (n=66)
Change from baseline at PBO week-12 (LOCF)	7.87	7.94	8.01	8.00	7.92	7.93
BL HbA <sub>1c</sub> (%)	8.74	8.92	8.70	8.82	8.74	8.99
BL FPG (mmol/L)	84.0	85.5	82.1	83.4	84.9	81.6
ΔHbA <sub>1c</sub> (%)	−0.27	−0.44	−0.62**	−0.69**	−0.77**	−0.83**
ΔFPG (mmol/L)	−0.50	−0.02*	−0.64	−1.04*	−0.94	−1.42**
ΔBW (kg)	−0.7	−1.6*	−1.9**	−2.2**	−2.6**	−2.8**
24h UGE (mmol)	16.9	217.9**	272.3**	346.2**	396.0**	402.9**
Difference from placebo, unadjusted p-value: *	<0.05, ** <0.01					

Clinical Trial Registration Number: NCT00800176

## PS 059 SGLT-2 IV

769

### Empagliflozin, a novel sodium glucose cotransporter-2 inhibitor, improves glucose homeostasis and preserves pancreatic beta cell mass in db/db mice

J. Jelsing<sup>1</sup>, E. Mayoux<sup>2</sup>, T. Klein<sup>2</sup>, R. Grempler<sup>2</sup>, M. Mark<sup>2</sup>;

<sup>1</sup>Gubra ApS, Hørsholm, Denmark, <sup>2</sup>Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany.

**Background and aims:** Empagliflozin is a potent and selective sodium glucose cotransporter-2 (SGLT2) inhibitor (SGLT-2) in development for the treatment of type 2 diabetes mellitus. This study investigated the effect of 12 weeks' treatment with empagliflozin on glucose homeostasis and pancreatic beta cell function in db/db mice.

**Materials and methods:** Db/db mice were treated with vehicle (n=10), empagliflozin 3 mg/kg (n=8) or empagliflozin 10 mg/kg (n=8) per os once daily for 84 days. Body weight, fasting plasma glucose, HbA<sub>1c</sub> and fasting insulin were measured. Following sacrifice, pancreatic tissue was dissected and total beta cell mass (insulin immunoreactive cells) and non-beta cell mass (glucagon, pancreatic polypeptide and somatostatin immunoreactive cells) were assessed using stereology.

**Results:** Treatment with empagliflozin 3 mg/kg and 10 mg/kg prevented the decline in body weight normally seen in diabetic db/db mice associated with health deterioration (+8.68g and +7.67g, respectively, versus -4.01g with vehicle;  $p < 0.05$ ). Empagliflozin resulted in a marked reduction in fasted plasma glucose (408mg/dl and 348mg/dl with 3 mg/kg and 10 mg/kg empagliflozin, respectively, versus 843mg/dl with controls at day 84 of treatment;  $p < 0.05$ ). The improvement in glucose homeostasis was confirmed by a reduction in HbA<sub>1c</sub> that reached significance for the 10 mg/kg dose (5.87% versus 4.71% with vehicle,  $p < 0.05$ ). Fasting insulin tended to be higher with empagliflozin 10 mg/kg compared with vehicle (31 versus 20  $\mu$ U/ml). In addition, at days 8 and 70 of treatment, results from oral glucose tolerance tests showed an improvement in glucose disposal rate with both empagliflozin 3 mg/kg and 10 mg/kg versus vehicle. The total beta cell mass was significantly larger for the 10 mg/kg empagliflozin group compared with vehicle (4.24mg versus 2.46mg,  $p < 0.05$ ), with a similar tendency in the 3 mg/kg group (3.29mg) compared to vehicle (NS). The non-beta-cell mass was the same across all three groups.

**Conclusion:** The data demonstrate that empagliflozin, by eliminating glucose in urine, improves glucose homeostasis and delays disease progression in db/db mice coupled to an improvement in beta cell mass.

Supported by: *Boehringer Ingelheim*

770

### The sodium glucose cotransporter-2 (SGLT-2) inhibitor empagliflozin lowers blood pressure independent of weight or HbA<sub>1c</sub> changes

T. Hach<sup>1</sup>, H.J. Lambers Heerspink<sup>2</sup>, E. Pfarr<sup>1</sup>, S. Lund<sup>1</sup>, L. Ley<sup>1</sup>, U.C. Broedl<sup>1</sup>, H.J. Woerle<sup>1</sup>;

<sup>1</sup>Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany,

<sup>2</sup>University Medical Center Groningen, University of Groningen, Netherlands.

**Background and aims:** Empagliflozin (EMPA) is a potent, selective sodium glucose cotransporter-2 inhibitor in development for treatment of type 2 diabetes mellitus (T2DM). Given evidence of effects of EMPA on glucose, weight and BP in Phase II trials, we analysed pooled patient data to evaluate the effects of EMPA on BP and the correlations between changes in weight or HbA<sub>1c</sub> and changes in BP.

**Materials and methods:** Two randomised, double-blind, parallel group, placebo (PBO)-controlled, dose-finding Phase 2b trials examined the safety and efficacy of EMPA as monotherapy (N=408) and as add-on to metformin (N=495) in patients with T2DM. Identical endpoints, study duration (12 weeks), and the sample size justified using the pooled dataset of the two EMPA doses tested in Phase 3 trials: EMPA 10 mg (N=152) and EMPA 25 mg (N=152) versus PBO (N=153). Changes from baseline in BP at 12 weeks were assessed and Pearson correlation coefficients were calculated. The frequency of any adverse events (AE) was calculated. Patients with a systolic BP (SBP) > 140 mmHg at baseline were analysed as a subgroup.

**Results:** Baseline mean ( $\pm$ SD) age, HbA<sub>1c</sub> and BMI of all patients in the Phase 2b trials were 57.9 $\pm$ 9.3 years, 7.9 $\pm$ 0.8% and 30.3 $\pm$ 4.7 kg/m<sup>2</sup>, respectively. Patients were predominantly white (83%), with an equal gender dis-

tribution and the majority (67%) were on anti-hypertensive medication at baseline. Baseline BP and changes from baseline in BP in patients on EMPA 10 mg, EMPA 25 mg and PBO are reported (Table). SBP decreased by 4-5 mmHg with EMPA; for each dose group, the change was significant versus placebo. The changes appeared to be larger in patients with SBP > 140 mmHg at baseline. DBP changes were more pronounced with EMPA, but did not reach statistical significance versus PBO. Changes in blood pressure did not correlate with changes in pulse rate. The Pearson correlation coefficients between change in weight and change in SBP were 0.10 (EMPA 10 mg), 0.04 (EMPA 25 mg) and 0.12 (PBO), and between change in HbA<sub>1c</sub> and change in SBP were -0.09 (EMPA 10 mg), -0.02 (EMPA 25 mg) and 0.11 (PBO). None of these correlations reached statistical significance ( $p > 0.14$ , for each). Including the number of antihypertensive medications at baseline in the ANCOVA model did not alter the PBO-adjusted effects on BP. The number of patients with AEs was comparable among treatment groups (34.2% in EMPA 10 mg, 31.6% in EMPA 25 mg, and 34.6% in PBO groups).

**Conclusion:** Treatment with EMPA was well tolerated and provided statistically significant and clinically meaningful reductions in SBP of 4-5 mmHg. Lowering of SBP with EMPA seemed more pronounced in patients with SBP > 140 mmHg at baseline. BP changes were not correlated with changes in weight or HbA<sub>1c</sub>, suggesting that EMPA has effects on BP related to its mode of action that are beyond its effects on weight and HbA<sub>1c</sub>.

Mean BP [mmHg]	Full Analysis Set			Patients with SBP > 140 mmHg		
	PBO (n=153)	EMPA 10 mg (n=152)	EMPA 25 mg (n=152)	PBO (n=33)	EMPA 10 mg (n=27)	EMPA 25 mg (n=38)
Baseline SBP $\pm$ SD	134.3 $\pm$ 15.9	131.3 $\pm$ 13.8	132.5 $\pm$ 14.6	157.2 $\pm$ 13.6	152.8 $\pm$ 8.9	151.1 $\pm$ 8.7
Change from baseline* in SBP $\pm$ SE	-1.2 $\pm$ 1.0	-3.8** $\pm$ 1.0	-4.5** $\pm$ 1.0	-10.4 $\pm$ 2.4	-17.0 $\pm$ 2.6	-13.4 $\pm$ 2.3
Baseline DBP $\pm$ SD	80.8 $\pm$ 8.4	79.1 $\pm$ 9.1	80.9 $\pm$ 9.2	86.2 $\pm$ 9.7	89.1 $\pm$ 9.6	88.4 $\pm$ 8.7
Change from baseline* in DBP $\pm$ SE	-1.8 $\pm$ 0.6	-2.3 $\pm$ 0.6	-2.7 $\pm$ 0.6	-6.1 $\pm$ 1.4	-8.1 $\pm$ 1.6	-7.6 $\pm$ 1.3

\*ANCOVA for LOCF with baseline BP, study and country \*\* $p < 0.05$  vs. PBO

Clinical Trial Registration Number: NCT00789035, NCT00749190

Supported by: *Boehringer Ingelheim*

771

### Effect of empagliflozin, on body weight, glucose control and plasma parameters in STZ induced diabetic rats fed a high-fat diet: comparison with exenatide

S.P. Vickers<sup>1</sup>, T. Klein<sup>2</sup>, R.B. Jones<sup>1</sup>, S.C. Cheetham<sup>1</sup>, E. Mayoux<sup>2</sup>, R. Grempler<sup>2</sup>, M. Mark<sup>2</sup>;

<sup>1</sup>RenaSci Ltd., Nottingham, UK, <sup>2</sup>Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany.

**Background and aims:** The effect of the sodium glucose cotransporter-2 inhibitor empagliflozin versus exenatide or vehicle on body weight, glucose control and relevant plasma parameters in animals fed a high fat diet and given low dose streptozotocin (STZ) to induce diabetes was determined.

**Materials and methods:** Male Sprague-Dawley rats (mean body weight approx. 430g) were administered vehicle or STZ (35 mg/kg ip) and maintained on a high fat diet (60% kcal as fat). After 1 week, these animals were allocated to one of 3 treatment groups (vehicle, empagliflozin, or exenatide; n=12 in all groups). After a 7-day baseline phase in which all animals received vehicle orally (po) once daily (qd), animals were dosed with vehicle (po) qd, empagliflozin (10 mg/kg po) qd or exenatide (30  $\mu$ g/kg/day) administered via subcutaneous osmotic pumps. Exenatide animals were also dosed with vehicle (po) qd. All other study animals were implanted with control pumps filled with saline. A non-STZ, vehicle-treated control group was also included (n=12). Body weight and food intake were monitored daily for 29 days. HbA<sub>1c</sub>, plasma glucose, insulin and triacylglycerol (TAG) were monitored. On Day 23, an OGTT (2 g/kg po) was performed after an overnight fast. Blood samples (4-hour fasted) were taken at termination (Day 29). Data were analysed by ANCOVA with multiple t tests for comparisons to control groups.

**Results:** At study end, STZ-treated animals exhibited a decrease in body weight (519 g vs 568 g;  $p < 0.001$ ) and plasma insulin (1.6 ng/ml vs 2.8 ng/ml;  $p < 0.01$ ) and an increase in HbA<sub>1c</sub> (9% vs 7%;  $p < 0.001$ ), plasma glucose (15.7

mM vs 8.3 mM;  $p<0.001$ ), and TAG (0.36 mM vs 0.2 mM;  $p<0.001$ ) vs vehicle-treated animals. In addition, STZ treatment significantly impaired glucose tolerance in an OGTT (AUC 52.2 mM.h vs 22.8 mM.h in vehicle-treated animals;  $p<0.001$ ). Empagliflozin had no statistically significant effect on body weight or food intake compared with STZ-treated controls, but improved the glucose control of diabetic rats in an OGTT (AUC 24.2 mM.h vs 52.2 mM.h;  $p<0.001$ ) and reduced overnight fasting glucose prior to the OGTT (6.61 mM vs 10.71 mM;  $p<0.001$ ). At Day 29, reductions in HbA1c (7.6% vs 9.0%;  $p<0.001$ ), plasma glucose (7.6 mM vs 15.7 mM;  $p<0.001$ ), insulin (0.7 ng/ml vs 1.6 ng/ml;  $p<0.01$ ) and TAG (0.21 mM vs 0.36 mM  $p<0.01$ ) were observed in empagliflozin-treated rats versus STZ-treated controls. In contrast, exenatide reduced body weight (8.2%;  $p<0.001$ ) and average daily food intake (15.9g vs 20.0g  $p<0.001$ ) but did not significantly improve glucose control in an OGTT ( $p=NS$ ) nor alter fasting glucose ( $p=NS$ ) versus STZ-treated control rats. Although chronic infusion of exenatide reduced plasma TAG (0.28 mM vs 0.36 mM  $p<0.05$ ) and glycerol (0.10 mM vs 0.18 mM;  $p<0.05$ ), HbA1c, plasma glucose and insulin were not significantly altered versus STZ-treated control rats.

**Conclusion:** Empagliflozin treatment significantly improved glucose control and HbA1c in an animal model in which insulin secretion was markedly impaired. In contrast, despite reducing body weight, exenatide did not improve overall glycaemic control. Significant efficacy with empagliflozin in this insulinopenic model of diabetes confirms the potential use of empagliflozin in the treatment of both type 1 and type 2 diabetes.

Supported by: Boehringer Ingelheim

## 772

### LX4211, a dual inhibitor of SGLT2 and SGLT1 enhances the effects of sitagliptin in patients with type 2 diabetes mellitus

B. Zambrowicz<sup>1</sup>, I. Ogbaa<sup>1</sup>, D. Powell<sup>1</sup>, P. Banks<sup>1</sup>, A. Turnage<sup>1</sup>, K. Frazier<sup>1</sup>, K.A. Boehm<sup>1</sup>, J. Freiman<sup>1</sup>, P. Lapuerta<sup>1</sup>, D. Ruff<sup>2</sup>, A. Sands<sup>1</sup>;

<sup>1</sup>Lexicon Pharmaceuticals, Inc., The Woodlands, <sup>2</sup>Healthcare Discoveries LLC d/b/a ICON Development Solutions, San Antonio, USA.

**Background and aims:** Combination therapy is often required in patients with type 2 diabetes mellitus (T2DM) to achieve glycemic control. LX4211 is a potent inhibitor of SGLT1 in the gastrointestinal (GI) tract and SGLT2 in the kidney. Local SGLT1 inhibition has been shown to stimulate secretion of GI hormones that play a role in glycemic control and appetite suppression. In this mechanistic study, we investigated the pharmacodynamic (PD) effects of the combination of LX4211 plus sitagliptin, a DPP-4 inhibitor, compared to either agent alone.

**Materials and methods:** 18 diabetic patients received single doses of either LX4211 (400 mg), sitagliptin (100 mg), or LX4211+sitagliptin on Days 1, 8, or 15 according to a balanced 3x3 crossover design with a 7-day washout period between each dosing day. PD parameters assessed included urinary glucose excretion (UGE), postprandial glucose (PPG), insulin, peptide YY (PYY), and total and active glucagon-like peptide 1 (GLP-1).

**Results:** All patients completed the study. There were no serious adverse events, deaths or discontinuations due to adverse events. The drug combination differed from sitagliptin for the PD parameters below. Data are presented as AUC<sub>0-last</sub> adjusted means, except for UGE.

**Conclusion:** LX4211 and sitagliptin, combined, significantly increased both active and total GLP-1 as compared to sitagliptin alone, and produced substantial reductions in PPG with lower endogenous insulin levels. LX4211 and sitagliptin appear to have complementary mechanisms of action that may provide a novel approach to combination therapy for T2DM.

PD Parameter	Treatment Group		
	LX4211	Sitagliptin	LX4211 + Sitagliptin
UGE (g/24hr)	92.2	7.5	83.9
Difference* from combo	-8.2 <sup>††</sup> (-24.8, 8.4)	76.4 (59.8, 93.0)	
Insulin (uM•hr/mL)	479.2	623.4	547.3
Difference* from combo	68.1 <sup>†</sup> (2.3, 133.9)	-76.1 <sup>†</sup> (-141.9, -10.3)	
PPG (mg•hr/L)	2366.7	2412.8	2209.6
Difference* from combo	-157.0 <sup>†</sup> (-312.9, -1.2)	-203.2 <sup>†</sup> (-359.0, -47.3)	
PYY (pmol•hr/L)	403.3	206.9	250.5
Difference* from combo	-152.8 <sup>††</sup> (-187.1, -118.5)	43.6 <sup>†</sup> (9.3, 77.9)	
GLP-1 total (pmol•hr/L)	161.9	105.9	135.8
Difference* from combo	-26.1 <sup>††</sup> (-38.8, -13.3)	29.9 <sup>††</sup> (17.2, 42.6)	
GLP-1 active (pmol•hr/L)	69.2	128.8	167.4
Difference* from combo	98.2 <sup>††</sup> (80.9, 115.4)	38.6 <sup>††</sup> (21.3, 55.9)	

\* estimated difference between combination and single dose drug adjusted means (95% CI)  
 p-values reflect differences in adjusted means between the combination of LX4211+Sitagliptin and each drug given as a single dose  
<sup>††</sup>  $p<0.001$ , <sup>†</sup>  $p<0.05$

Clinical Trial Registration Number: NCT01441232

## 773

### LX4211, a dual SGLT1/SGLT2 inhibitor shows a favourable gastrointestinal and genitourinary safety profile in type 2 diabetes mellitus patients and healthy subjects

J. Freiman, G.-L. Ye, I. Ogbaa, K.A. Boehm, A. Turnage, K. Frazier, A. Sands, B. Zambrowicz;

Lexicon Pharmaceuticals, Inc., The Woodlands, USA.

**Background and aims:** Selective SGLT2 inhibitors are designed to treat T2DM by reducing glucose reabsorption by the kidney, resulting in urinary glucose excretion. An increased incidence of genitourinary (GU) infections has been observed with these agents. Because patients with genetic mutations in SGLT1, that completely inhibit SGLT1 function, experience glucose-galactose malabsorption, most SGLT inhibitors selectively inhibit SGLT2 to avoid the theoretical risk of diarrhea resulting from pharmacologic inhibition of SGLT1 inhibition. In clinical trials to date, LX4211, a potent dual inhibitor of SGLT1 and SGLT2, has shown both significantly improved glycemic parameters and a favorable gastrointestinal (GI) safety profile. This overview of LX4211 clinical studies evaluates the occurrence of adverse events (AEs) that may be associated with the pharmacologic action of LX4211.

**Materials and methods:** LX4211 was studied at single and multiple doses in healthy subjects and patients with T2DM (Figure 1) at doses ranging from 5 to 500 mg/day. LX4211 safety was assessed through AEs collected from the first dose to 30 days after the last dose. Relevant AEs were reviewed for each study.

**Results:** Of the 12 AEs of diarrhea, 5 occurred on LX4211 out of 1090 person days (PD), 3 with metformin out of 18 PD, and 4 with LX4211+metformin out of 18 PD. All resolved within 1 day and were generally mild. There were no episodes of hypoglycemia, with no deaths, serious AEs, or AEs leading to discontinuation. No GU infection was reported in any LX4211-treated subject.

**Conclusion:** In clinical studies to date, LX4211 is well-tolerated with no evidence of clinically significant hypoglycemia, diarrhea, or GU infections.



Overview of Adverse Events<sup>a</sup>

Dose, n	Diarrhea	Constipation	Nausea	Vomiting
<b>Gastrointestinal</b>				
<b>Healthy Subjects</b>				
<b>LX4211.101</b> - Placebo-controlled, double-blind, randomized, single and multiple ascending-dose study				
Placebo, n=24	0	0	1	0
5-100 mg, n=42	1	0	1	1
150-500 mg, n=30	0	0	0	0
<b>LX4211.103</b> - Open-label, randomized, single-dose (3 treatment), crossover study of LX4211 and metformin				
400 mg, n=18	1	1	0	0
Metformin 1000 mg, n=18	3	1	0	0
LX4211 + Metformin, n=18	4	0	1	0
<b>LX4211.104</b> - Placebo-controlled, double-blind, randomized, multiple-dose study of LX4211 relative to meals				
Placebo, n=2	0	0	0	0
400 mg, n=10	0	0	0	0
<b>Type 2 Diabetic Patients</b>				
<b>LX4211.102</b> - Open-label, randomized, 3-way crossover study of 2 oral formulations of LX4211 in T2DM				
Tablet (2 x 150 mg), n=12	0	1	0	0
Tablet (6 x 50 mg), n=12	0	0	0	0
Oral solution (300 mg), n=12	1	2	0	0
<b>LX4211.201</b> - Placebo-controlled, double-blind, randomized, 28-day study in T2DM				
Placebo, n=12	0	2	2	1
150 mg, n=12	1	3	2	1
300 mg, n=12	1	2	1	0
<b>No urinary or genital AEs were reported.</b>				
<sup>a</sup> Data are presented as number of subjects experiencing the AE.				

## 774

**LX4211 increases serum GLP-1 and PYY levels after oral glucose challenge in mice by inhibiting SGLT1-mediated intestinal glucose absorption**

D. Powell, M. Smith, S. Zhao, J. Greer, A. Harris, C. DaCosta, A. Sands, B. Zambrowicz, Z.-M. Ding;  
Lexicon Pharmaceuticals, Inc., The Woodlands, USA.

**Background and aims:** LX4211, a dual SGLT1/SGLT2 inhibitor, is designed to reduce renal glucose reabsorption by inhibiting SGLT2 and intestinal glucose absorption by inhibiting SGLT1. Increased intestinal glucose levels secondary to SGLT1 inhibition should trigger release of intestinal hormones such as glucagon-like peptide (GLP)-1 and peptide YY (PYY). We asked if oral glucose increases levels of cecal glucose and serum GLP-1 and PYY in mice treated with LX4211, and if these findings are recapitulated in SGLT1 or SGLT2 knockout (KO) mice.

**Materials and methods:** Study 1: Wild-type (WT) mice raised on glucose-containing diet received vehicle or LX4211 (60 mg/kg) by gavage; 30 min later these mice, along with SGLT1 and SGLT2 KO and WT littermate mice raised on glucose-free diet, received a glucose-containing meal (9.2 g glucose/kg, 2.5 g protein/kg, 0.6 g fat/kg) by gavage and were studied 3 hr later. Study 2: 30 min after WT mice received vehicle or LX4211 (60 mg/kg) by gavage, they were gavaged different doses of glucose (N = 5/group); total GLP-1 AUC (nM x min) was then estimated over the next 6 hr, and data were analyzed by ANOVA followed by Bonferroni-Dunn post-hoc test.

**Results:** The results for Study 1 are presented in Table 1.

Table 1

Mice	Cecal G (mg)	Total GLP-1 (pM)	Active GLP-1 (pM)	PYY (ng/ml)
WT + Vehicle	1.2 ± 0.4	55 ± 2	7.2 ± 0.6	0.9 ± 0.1
WT + LX4211	18.9 ± 2.2***	161 ± 15***	16.3 ± 2.1***	2.2 ± 0.3**
SGLT1 WT	0.1 ± 0.1	36 ± 4	2.7 ± 0.2	1.0 ± 0.1
SGLT1 KO	16.7 ± 5.4*	158 ± 14***	12.5 ± 3.8*	2.2 ± 0.6*
SGLT2 WT	0.7 ± 0.5	52 ± 2	7.0 ± 0.1	1.6 ± 0.1
SGLT2 KO	0.7 ± 0.4	53 ± 4	7.1 ± 0.4	1.3 ± 0.1

Values are mean ± SE; N > 4; unpaired t test. \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001

For Study 2, Total GLP-1 AUC (mean ± SE) was 18 ± 3 (vehicle) vs 42 ± 4 (LX4211) after 2 g glucose/kg (p > 0.05), 19 ± 1 (vehicle) vs 59 ± 6 (LX4211) after 4 g glucose/kg (p > 0.05), 29 ± 4 (vehicle) vs 84 ± 12 (LX4211) after 6 g glucose/kg (p < 0.01), and 25 ± 4 (vehicle) vs 160 ± 36 (LX4211) after 8 g glucose/kg (p < 0.001).

**Conclusion:** Oral glucose increased levels of intestinal glucose and serum GLP-1 and PYY in LX4211-treated mice, findings that were recapitulated in SGLT1, but not SGLT2, KO mice. These data suggest that LX4211 increases serum GLP-1 and PYY levels after an oral glucose challenge by inhibiting intestinal SGLT1.

## 775

**LX4211, a dual inhibitor of SGLT1 and SGLT2, results in postprandial glucose reductions in healthy subjects**

P. Lapuerta<sup>1</sup>, I. Ogbaa<sup>1</sup>, D. Powell<sup>1</sup>, P. Banks<sup>1</sup>, A. Turnage<sup>1</sup>, K. Frazier<sup>1</sup>, K.A. Boehm<sup>1</sup>, J. Freiman<sup>1</sup>, D. Ruff<sup>2</sup>, A. Sands<sup>1</sup>, B. Zambrowicz<sup>1</sup>;

<sup>1</sup>Lexicon Pharmaceuticals, Inc., The Woodlands, <sup>2</sup>Healthcare Discoveries LLC d/b/a ICON Development Solutions, San Antonio, USA.

**Background and aims:** SGLT2 is the target of several investigational compounds that aim to treat type 2 diabetes mellitus (T2DM). LX4211 is a potent systemic inhibitor of SGLT2 that also inhibits SGLT1 locally in the gastrointestinal (GI) tract. In prior clinical studies, dual inhibition by LX4211 provided glycemic control and metabolic benefits without triggering clinically significant GI side effects. In this study we explored the impact of timing of the dosing regimen on a variety of pharmacodynamic (PD) parameters including postprandial glucose (PPG), fasting plasma glucose (FPG), and insulin.

**Materials and methods:** 12 healthy subjects were enrolled, sequestered, and randomly assigned to LX4211 (n=10) or placebo (n=2). Subjects received LX4211 two (2) hours prior to breakfast for 7 days to establish a steady state, followed by dosing at 5 different times relative to meal, in a Latin Square design balanced for first order carryover effects, on Days 8-12. PD parameters, including PPG, FPG, and insulin were assessed. Safety and tolerability were also evaluated throughout the study.

**Results:** All 12 subjects completed the study. Results across dosing times were comparable. All adverse events (AE) were mild and infrequent; no events were deemed to be related to the administration of LX4211.

**Conclusion:** LX4211 produced marked suppression of postprandial glucose excursion and hyperinsulinemia after breakfast with either morning or split dose, without producing hypoglycemia or diarrhea in healthy subjects.

Days 8-12, change from baseline (Day -1):

Dose Schedule→ PD Variable↓	Immediately prior to breakfast	Split dose <sup>a</sup> (AM/PM)
FPG (mg/dL) (95% CL)	-6.12 (-8.26, -3.98)†	-6.68 (-8.81, -4.54)†
PPG (mg•hr/dL) AUC <sub>0-last</sub> (95% CL) <sup>b</sup>	-74.71† (-99.75, -49.67)	-103.19† (-128.23, -78.15)
Insulin (μU•hr/mL) AUC <sub>0-last</sub> (95% CL) <sup>b</sup>	-193.71† (-189.51, -134.14)	-163.65† (-191.33, -135.97)

† p < 0.001 of within schedule comparison vs Day -1

<sup>a</sup> Split dose was taken 1 hour prior to both breakfast and dinner

<sup>b</sup> AUC<sub>0-last</sub> was calculated from 0-minutes to 13-hours postdose

Clinical Trial Registration Number: NCT01334242

## 776

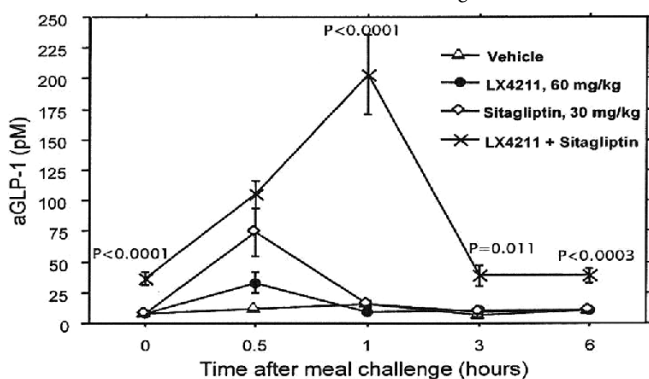
### The combination of LX4211 and DPP4 inhibition synergistically increases serum levels of active GLP-1 after glucose challenge in mice

Z.-M. Ding, M. Smith, S. Zhao, J. Greer, A. Harris, A. Sands, B. Zambrowicz, D. Powell;  
Lexicon Pharmaceuticals, Inc., The Woodlands, USA.

**Background and aims:** LX4211, a dual SGLT1/SGLT2 inhibitor, increases serum levels of total GLP-1 and active GLP-1 (aGLP-1) after an oral glucose challenge. These increases result from inhibition of intestinal SGLT1 by LX4211, which delays small intestinal glucose absorption and triggers increased release of GLP-1 by the gastrointestinal tract. Sitagliptin is a DPP4 inhibitor that increases aGLP-1 levels by inhibiting DPP4-mediated aGLP-1 inactivation. We asked whether the combination of LX4211 and Sitagliptin would increase aGLP-1 levels more than either compound alone after oral glucose challenge.

**Materials and methods:** Male C57BL/6J mice, 27 weeks of age and fed 45% lard diet from weaning, received either vehicle, LX4211 (60 mg/kg), Sitagliptin (30 mg/kg), or both LX4211 and Sitagliptin by oral gavage for 14 days. 30 minutes after their last dose, these mice received a glucose-containing meal (9.2 g glucose/kg, 2.5 g protein/kg, 0.6 g fat/kg) by oral gavage. Serum aGLP-1 levels were measured on samples obtained at baseline and at 0.5-, 1-, 3- and 6-hours after glucose gavage using the Millipore aGLP-1 ELISA kit. Data were analyzed by 2-way ANOVA.

**Results:** As shown in the figure below, a significant interaction of LX4211 and Sitagliptin was demonstrated at baseline and at 1-, 3- and 6-hours post glucose challenge, indicating a synergistic increase in aGLP-1 with the combination of LX4211 and Sitagliptin compared to effects seen with each compound alone. Four additional studies confirmed these findings.



**Conclusion:** These data suggest that SGLT1 inhibition by LX4211 and DPP-4 inhibition by Sitagliptin increase aGLP-1 by complementary mechanisms of action which, when combined, lead to a synergistic increase in aGLP-1 levels that may be advantageous to patients with T2DM.

## 777

### Health-related quality of life (EQ-5D) among type 2 diabetes mellitus patients treated with dapagliflozin for 24 weeks

A. Ingelgård<sup>1</sup>, S. Grandy<sup>2</sup>, A. Langkilde<sup>1</sup>, J.E. Sugg<sup>2</sup>, S.J. Parikh<sup>2</sup>;  
<sup>1</sup>AstraZeneca, Mölndal, Sweden, <sup>2</sup>AstraZeneca, Wilmington, USA.

**Background and aims:** Dapagliflozin is a first in class, oral sodium-glucose co-transporter 2 (SGLT2) inhibitor, in clinical development for the treatment of type 2 diabetes mellitus (T2DM). Dapagliflozin lowers blood glucose by increasing urinary glucose excretion and is associated with improvements in HbA1c and reductions in body weight. This study evaluated the health status and health-related quality of life (HRQOL) among T2DM patients treated with dapagliflozin in a randomised clinical trial.

**Materials and methods:** Subjects with T2DM (BMI  $\geq 25$  kg/m<sup>2</sup>; men 56%, women 44%; mean age, 61 years) who had inadequate glycaemic control on metformin (MET) alone were enrolled in a 24-week, international, double-blind, randomised, placebo-controlled study to evaluate the effect of dapagliflozin in combination with MET on total body weight. Subjects completed the EQ-5D questionnaire at baseline and at week 24. Subjects treated with dapagliflozin 10 mg + MET (n = 87) were compared with subjects treated with placebo + MET (n = 89), based on an ANCOVA model with treatment group and gender as effects and baseline value as a covariate.

**Results:** EQ-5D index baseline means (SD) were 0.85 (0.16) and 0.82 (0.15) for dapagliflozin and placebo, respectively. Corresponding 24-week values were 0.88 (0.17) and 0.87 (0.16), respectively. The ANCOVA model indicated no difference (-0.01; CI [-0.05, 0.03]; p-value 0.49). EQ-5D visual analog scale (VAS) baseline means (SD) were 72.8 (19.39) and 73.7 (15.49) for dapagliflozin and placebo, respectively. Corresponding 24-week values were 77.4 (15.21) and 78.3 (10.65), respectively. The ANCOVA model indicated no difference (-0.6; CI [-3.9, 2.8]; p-value 0.74).

**Conclusion:** Results indicated that the patients maintained high HRQOL scores from baseline to week 24 in both treatment groups as measured by EQ-5D index and VAS. Health status and HRQOL were maintained at a high level during treatment with dapagliflozin, a novel, selective SGLT2 inhibitor.

Clinical Trial Registration Number: NCT00855166

Supported by: BMS and AZ

## PS 060 Incretins and atherosclerosis

778

### The anti-atherosclerosis effect of dipeptidyl peptidase-4 inhibitor is mainly attributable to increased incretins in apolipoprotein-E null mice

T. Hirano, M. Terasaki, M. Nagashima, Y. Nogi, K. Notomi;  
Diabetes, Metabolism, and Endocrinology, Showa University School of Medicine, Tokyo, Japan.

**Background and aims:** Several recent reports have revealed that dipeptidyl peptidase (DPP)-4 inhibitor suppresses atherosclerosis in hypercholesterolemic mice. It remains to be seen, however, whether this effect is mainly attributable to increased active incretins; glucagon-like peptide-1 (GLP-1) and/or glucose-dependent insulintropic polypeptide (GIP).

**Materials and methods:** Seventeen-week-old apolipoprotein E-null (*ApoE*<sup>-/-</sup>) mice fed an atherogenic diet were administered a DPP-4 inhibitor, vildagliptin (100 micromol/kg/day), in drinking water for 4 weeks. During vildagliptin administration, the animals were subcutaneously infused (via osmotic minipumps) with either saline, a GLP-1 receptor (R) blocker (Exendin-9, 22 nmol kg<sup>-1</sup> day<sup>-1</sup>), a GIPR blocker ((Pro3) GIP, 25 nmol kg<sup>-1</sup> day<sup>-1</sup>), or both. Aortic atherosclerosis and oxidized-low-density-lipoprotein-induced foam cell formation in exudate peritoneal macrophages were determined. The direct effect of mouse DPP-4 (CD26: 100–500 ng/ml) or DPP-4 inhibitor (PKF-275-055: 20 micromol) on foam cell formation was also determined.

**Results:** Orally administered vildagliptin increased plasma levels of active GLP-1 by 3.5-fold without affecting food intake, body weight, blood pressure, or plasma parameters (excluding a mild cholesterol reduction). Vildagliptin significantly suppressed total aortic atherosclerotic lesions, atheromatous plaque in the aortic root, and macrophage accumulation in the aortic wall (60% reduction,  $p < 0.0001$ ). Likewise, vildagliptin suppressed foam cell formation by 40% in exudate peritoneal macrophages. Exendin-9 or (Pro3)GIP partially attenuated the vildagliptin-induced suppression of atherosclerosis and macrophage foam cell formation, whereas the combination of both receptor blockers virtually abolished both of these vildagliptin effects. DPP-4 or DPP-4 inhibitor did not affect the foam cell formation *in vitro*.

**Conclusion:** DPP-4 inhibitor substantially suppresses atherosclerosis in *ApoE*<sup>-/-</sup> mice. This suppressive effect is mainly attributable to increased levels of both GLP-1 and GIP, but not to the inactivation of DPP-4.

779

### Improved endothelial cell function and inflammation after replacement of insulin by liraglutide in combination with metformin and sulfonylurea in type 2 diabetic patients

T. Kamada<sup>1</sup>, E. Tanoue<sup>1</sup>, T. Harada<sup>1</sup>, M. Kurano<sup>1</sup>, C. Hisadome<sup>1</sup>, T. Kamikubo<sup>2</sup>, H. Sameshima<sup>2</sup>;

<sup>1</sup>Diabetes, Imamura Bun-in Hospital, <sup>2</sup>Nanami Clinic, Kagoshima, Japan.

**Background and aims:** Glucagon-like peptide-1 (GLP-1) receptor agonists improve glycemic control in type 2 diabetic patients (T2DM), being associated with weight loss and a low risk of hypoglycemia. They are also suggested to have a beneficial effect on myocardium, endothelium and vasculature, as well as potential anti-inflammatory and antiatherogenic actions. In diabetes, both impaired endothelial cell function and inflammatory condition are considered important in the progression of the disease itself and atherosclerosis. We studied the effect of replacement of insulin by liraglutide on endothelial cell function and inflammatory biomarker in T2DM treated with insulin in combination with oral hypoglycemics.

**Materials and methods:** Thirty-three T2DM patients (male 14, female 19; age: 56±12, BMI: 26.1±4.2) were enrolled to study. They were treated with insulin (total daily dose: 20.2±11.7 (5–46) U) in combination with metformin and sulfonylurea. Insulin was replaced by liraglutide 0.9mg, and clinical parameters and post ischemic flow-mediated dilatation (FMD) of brachial artery were evaluated after 3 and 6 months. The clinical parameters included HbA1c, 1,5-anhydroglucitol (1,5AG): a marker of postprandial hyperglycemia, serum C-peptide (sCP), high sensitivity C-reactive protein (hsCRP) and estimated GFR (eGFR). The FMD was measured in fasting state. Foods containing caffeine and smoking were prohibited for 10hrs before the measurement. ANOVA and Freedman plus Wilcoxon tests were performed for statistical analysis.

**Results:** 1) HbA1c was significantly improved after replacement of insulin by liraglutide for 3 and 6 months (from 7.88±0.95 to 7.19±0.0.90 and 7.50±0.91%,

$p < 0.001$  and  $p < 0.006$  vs. before) without hypoglycemic episode. The improvement was more apparent in the patients having higher sCP levels, less daily insulin doses. 2) The changes of fasting blood glucose and 1,5AG were not statistically significant after 3 and 6 months. 3) Average body weight was decreased -2kg after 3 months and -1Kg after 6 months as compared before. 4) FMD was significantly improved after 6 months (from 4.56±2.22 to 4.70±2.42 and 5.92±2.51%;  $p < 0.05$  vs. before). The proportion of patients FMD above cutoff value 6% was 24% of total before the replacement, but it increased up to 54% after 6 months liraglutide treatment. The improvement of FMD was independent of either weight loss or changes of blood glucose (HbA1c, FBS and 1,5AG). 5) Blood pressure was not altered after the replacement, but eGFR were significantly increased after 6 months (from 74.1±11.3 to 77.4±12.5 ml/min/1.73m<sup>2</sup>,  $p < 0.05$ ). 7) HsCRP was significantly decreased after 6 months as compared before (from 0.12±0.15 to 0.04±0.04mg/l,  $p < 0.05$ ).

**Conclusion:** Replacement of insulin by liraglutide in combination with metformin and sulfonylurea improved HbA1c and body weight in T2DM patients without hypoglycemia. Impaired FMD in T2DM was recovered after liraglutide treatment, which might be brought about by its direct effect on endothelial function. Treatment with liraglutide also decreased hsCRP, a marker of inflammation. Our results suggest that replacement of insulin by liraglutide might be beneficial for the treatment of vasculopathy of T2DM.

780

### The GLP-1 receptor agonist liraglutide attenuates atherosclerotic lesion development and enhances plaque stability in an ApoE<sup>-/-</sup> mouse model

A.E. Dear<sup>1</sup>, T. Gaspari<sup>2</sup>, I. Welungoda<sup>2</sup>, R.E. Widdop<sup>2</sup>, R.W. Simpson<sup>3</sup>;

<sup>1</sup>Medicine, <sup>2</sup>Pharmacology, <sup>3</sup>Endocrinology, Monash University, Melbourne, Australia.

**Background and aims:** The once daily GLP-1 receptor (GLP-1R) agonist, liraglutide, has been approved as a new treatment for type 2 diabetes. Liraglutide improves glycaemic control, lowers body weight and is the subject of clinical trials to evaluate effects on cardiovascular disease. We have previously demonstrated liraglutide-mediated attenuation of tumour necrosis factor alpha (TNFα) induced plasminogen activator inhibitor type 1 (PAI-1) and vascular cell adhesion molecule expression in human vascular endothelial cells *in vitro* and significant improvement in endothelial function and attenuation of adhesion molecule expression *in vivo* in the ApoE<sup>-/-</sup> mouse model. The current study aimed to determine the *in vivo* effect of liraglutide on atherosclerotic plaque formation and stability in the ApoE<sup>-/-</sup> mouse model.

**Materials and methods:** *In vivo* experiments utilized early onset disease (1) or established disease (2) protocols: (1) 17 week old ApoE<sup>-/-</sup> mice maintained on a normal chow diet were simultaneously commenced on a high fat diet and either saline (vehicle), liraglutide (300µg/kg twice daily, s.c.) or liraglutide + exendin-9 (150pmol/kg/min s.c., osmotic mini-pump) for 4 weeks or (2) 18 week old ApoE<sup>-/-</sup> mice maintained on a high fat diet for 12 weeks were treated with a regime identical to protocol 1 for 4 weeks.

**Results:** Oil red O staining and intima to media ratio (IMR) analysis of atherosclerotic plaques from the aorta and brachiocephalic artery (BCA) identified a significant reduction in lipid deposition and IMR in liraglutide treated mice from protocol 1. A statistically significant increase in vascular smooth muscle cell (SMC) content, as assessed by α-smooth muscle actin staining, of BCA atherosclerotic lesions in liraglutide-treated mice from protocol 1 was identified. The increase in SMC composition and decrease in lipid deposition in plaques from the BCA of protocol 1 mice are suggestive of a plaque stabilising effect associated with liraglutide treatment. Attenuation of the liraglutide-mediated plaque stabilising effect by concurrent administration of the GLP-1R antagonist exendin-9 confirmed the GLP-1R-dependence of this effect. Vascular reactivity studies identified no apparent endothelial dysfunction in the aorta of mice from protocol 1 however liraglutide treatment significantly attenuated the endothelial dysfunction in mice from protocol 2.

**Conclusion:** Together these results suggest a potential therapeutic benefit of GLP-1 receptor activation by liraglutide in the attenuation of early atherogenesis and stabilisation of existing atherosclerotic disease.

Supported by: Novo Nordisk Research Grant



## 781

**Long-term administration of exenatide and changes in body weight and markers of cardiovascular risk: a comparative study with glimepiride**

R. Simo<sup>1</sup>, B. Guerci<sup>2</sup>, G. Schernthaner<sup>3</sup>, B. Gallwitz<sup>4</sup>, J. Guzman<sup>5</sup>, F. Dotta<sup>6</sup>, A. Festa<sup>7</sup>, H. Sapin<sup>8</sup>, S. Chen<sup>9</sup>, J. Kiljanski<sup>10</sup>;

<sup>1</sup>Vall d'Hebron Research Institute, Barcelona, Spain, <sup>2</sup>Brabo Hospital & CIC, Nancy, France, <sup>3</sup>Rudolfstiftung Hospital, Vienna, Austria, <sup>4</sup>Eberhard Karls University, Tübingen, Germany, <sup>5</sup>Celaya Center for Specialist Medicine, Guanajuato, Mexico, <sup>6</sup>Policlinico Le Scotte, Siena, Italy, <sup>7</sup>Lilly Austria, Vienna, Austria, <sup>8</sup>Lilly France, Paris, France, <sup>9</sup>Amylin Pharmaceuticals, Inc., San Diego, USA, <sup>10</sup>Lilly Polska, Warsaw, Poland.

**Background and aims:** Type 2 diabetes is associated with elevated risk of cardiovascular (CV) events and it is important to examine whether anti-diabetes medications affect CV risk. Data relating to CV risk factors were examined from the EUROpan EXenAtide (EUREXA) clinical study, which compared glycaemic control during treatment with exenatide twice daily (EXE) versus glimepiride once daily (GLI), added to metformin.

**Materials and methods:** In the long-term, randomised, parallel-group, open-label study, patients with inadequate glycaemic control with metformin alone received add-on EXE (N=511) or GLI (N=508). Patients with inadequate HbA1c with EXE or GLI (HbA1c >9% at any visit or >7% at 2 successive visits, excluding first follow-up visit) were discontinued (EXE n=203, GLI n=262) and offered alternative therapy. Body weight, waist circumference, lipid profiles, blood pressure (BP), and high sensitivity C-reactive protein (hsCRP) concentration were evaluated throughout, with analysis using mixed model repeated measures with terms for baseline value, treatment, visit and interaction.

**Results:** Patients had overall baseline mean age 56 y, diabetes duration 6 y, BMI 32.5 kg/m<sup>2</sup> and HbA1c 7.4%. Mean (SD) baseline weight was 92.6 (16.6) and 90.9 (15.1) kg in EXE and GLI treatment groups, respectively; weight decreased by -3.4 (3.9) kg with EXE and increased by +1.0 (2.9) kg with GLI at 6 mo, with a significant between-group difference from 6 mo to 3 y (3 y difference -3.7 kg, *P*<0.001). Baseline waist circumference was 108.3 (11.8) and 107.6 (11.5) cm in EXE and GLI groups, respectively, which decreased by -2.7 (4.7) cm with EXE and changed by +0.2 (3.8) cm with GLI at 6 mo, with a significant between-group difference maintained from 6 mo (difference -2.8 cm, *P*<0.001) to 3 y (-4.5 cm, *P*<0.001). Baseline systolic BP was 132.9 (15.7) and 133.6 (15.4) mmHg, respectively, which decreased with EXE and was unchanged with GLI, with a between-group difference from 6 mo (-3.6 mmHg, *P*<0.001) to 3 y (-5.2 mmHg, *P*<0.001). Serum total and LDL cholesterol did not differ between groups. Mean baseline HDL cholesterol was 1.26 (0.30) and 1.25 (0.32) mmol/L, respectively, and was increased to a greater extent with EXE than with GLI at 1 y (difference +0.05 mmol/L, *P*=0.002) and 3 y (+0.06 mmol/L, *P*=0.001). Mean baseline triglyceride was 1.95 (1.19) and 2.02 (1.29) mmol/L, respectively, and was decreased to a greater extent with EXE than with GLI at 2 y (difference -0.20 mmol/L, *P*=0.017) and 3 y (-0.26 mmol/L, *P*=0.004). Baseline hsCRP was 4.8 (7.7) and 4.2 (5.1) mg/L, respectively, and decreased with EXE but not GLI, with a between-group difference from 1 y (-1.3 mg/L, *P*=0.011) to 3 y (-1.2 mg/L, *P*=0.004).

**Conclusion:** In patients with type 2 diabetes randomised to EXE in the EUREXA study, cardiovascular risk factors, including weight, BP, and hsCRP, were significantly improved compared with those for patients randomised to GLI, as add-on to metformin. The differences between EXE- and GLI-treated patients were apparent from 1 y through to 3 y of treatment.

Clinical Trial Registration Number: NCT00359762

## 782

**Effects of liraglutide on plasma ghrelin concentrations and oxidative stress in patients with type 2 diabetes: a 2-month prospective pilot study**

M. Rizzo<sup>1,2</sup>, G. Li Volti<sup>3,2</sup>, A. Patti<sup>1</sup>, I. Barbagallo<sup>3</sup>, V. Di Bartolo<sup>1</sup>, V. Giglio<sup>1</sup>, A. Tamburello<sup>1</sup>, A. Zabbara<sup>1</sup>, N. Abate<sup>4</sup>, G. Montalto<sup>1</sup>;

<sup>1</sup>University of Palermo, Italy, <sup>2</sup>Euro-Mediterranean Institute of Science and Technology, Palermo, Italy, <sup>3</sup>University of Catania, Italy, <sup>4</sup>Division of Endocrinology, The University of Texas Medical Branch, Galveston, USA.

**Background and aims:** Liraglutide is an effective hypoglycemic agent recently approved in Italy for clinical use in patients with type 2 diabetes. Since cardiovascular disease is the main cause of morbidity and mortality in this patient population, it is important to assess the effects of liraglutide on atherogenesis. To this goal, we evaluated the effects of intervention with liraglutide on glucose-independent mechanisms of atherogenesis. Since recent findings

suggest that plasma ghrelin concentrations may be affected by liraglutide and that ghrelin may play an important anti-atherogenic role, we hypothesized that liraglutide could have an effect on plasma ghrelin concentrations. We also evaluated whether these glucose-independent metabolic effects of liraglutide would include reduction in oxidative stress, a major contributor to atherogenesis.

**Materials and methods:** Twenty patients with type 2 diabetes (10 men and 10 women, age: 57±13 years) were enrolled in a prospective pilot study to assess the effect of combined therapy with liraglutide (0.6mg/daily for the first 2 weeks, followed by a dose of 1.2mg/daily) and metformin (1500mg/daily) on plasma ghrelin concentrations (measured by ELISA) and oxidative stress status, measured by determination of reactive oxygen species (using a fluorescent probe 2,7-dichlorofluorescein), serum lipid hydroperoxides (following the oxidation of Fe<sup>2+</sup> to Fe<sup>3+</sup> in the presence of xylenol orange) and serum total thiol groups (in 200 µl of serum, using a spectrophotometric assay based on the reaction of thiol groups with 2,2-dithio-bis-nitrobenzoic acid). Patients were newly diagnosed or previously treated subjects on stable doses of oral hypoglycemic agents. All samples were obtained in the morning, after a 16-18 hours fasting. Statistical analysis was performed using non-parametric Wilcoxon paired test and the Spearman correlation method.

**Results:** At baseline patients had a body-mass-index (BMI) of 28.7±5.3, with average fasting glucose 8.7±3.6 mmol/L and HbA1c 8.0±2.0%. The following changes were recorded after 2 months of therapy: BMI decreased to 28.5±4.3 (*p*=0.9), fasting glucose decreased to 7.6±2.3 mmol/L (*p*=0.0277), and HbA1c to 7.4±1.6% (*p*=0.0378). Plasma ghrelin concentrations increased from 8.2±4.1 to 13.6±7.7 pg/ml (*p*=0.0018), while serum lipid hydroperoxides were reduced from 0.11±0.05 to 0.04±0.07 pg/ml (*p*=0.0283). No significant differences were found in the reactive oxygen species or total thiol groups. Furthermore, changes in plasma ghrelin and lipid hydroperoxides did not correlate with changes in BMI, fasting glucose or HbA1c.

**Conclusion:** Liraglutide has favorable effects on plasma ghrelin concentrations and serum lipid hydroperoxides, both markers of cardio-metabolic risk, in patients with type-2 diabetes after only 2 months of therapy. These data seem not to be related with the known liraglutide effects on plasma glucose concentrations.

## 783

**Exenatide therapy reduced intima-media thickness of carotid artery in patients with type 2 diabetes**

M. Yoshida<sup>1</sup>, N. Yamamoto<sup>1</sup>, A. Saeki<sup>1</sup>, M. Sugino<sup>1</sup>, T. Kuzuya<sup>2</sup>, N. Ohsawa<sup>2</sup>, S. Yoshida<sup>3</sup>, H. Sano<sup>4</sup>, T. Hanafusa<sup>4</sup>;

<sup>1</sup>Internal Medicine, Aino Hospital, Ibaraki City, <sup>2</sup>Aino Institute for Aging Research, Ibaraki City, <sup>3</sup>Yoshida Clinic, Otsu City, <sup>4</sup>Internal Medicine 1, Osaka Medical College, Takatsuki City, Japan.

**Background and aims:** In the treatment of type 2 diabetes, maintenance of good glycemic control including post prandial hyperglycemia from early stage is important for the suppression of cardiovascular events. However, it is not easy by conventional treatments to maintain a long-term good glycemic control while avoiding weight gain. GLP-1 receptor agonist exenatide is expected not only to improve glycemic control but also to exhibit antiatherosclerotic effect. The antiatherosclerotic effects of exenatide were reported in some animal models, but the reports in human are rare. The intima-media thickness (IMT) by ultrasound evaluation of carotid artery is known to be correlated with stroke or myocardial infarction and is a good indicator for arteriosclerosis. This study is aimed to evaluate the efficacy of exenatide for prevention of arteriosclerosis in patients with type 2 diabetes.

**Materials and methods:** The subjects of exenatide group were 56 outpatients with type 2 diabetes (25 men and 31 women), aged 63.8±11.0 years old, BMI 27.7±6.1, and with HbA1c higher than 6.9%, or below 6.9% but with repeating hypoglycemia, by past treatment with OHA and/or insulin. The control group was 50 age-, HbA1c- and BMI-matched subjects with type 2 diabetes. In the exenatide group, exenatide was prescribed in addition to the previous treatment for 12 months, 5 mcg at first, and 10 mcg after 1 month, twice daily in the morning and evening. In sulfonylurea users, the dose of sulfonylurea was reduced to prevent unwanted hypoglycemia. We measured max and mean IMTs of carotid artery with the standardized method using ultrasonography.

**Results:** Three patients discontinued the use of exenatide because of nausea. After the start of exenatide, max IMT was significantly decreased from 2.07±0.79 to 1.91±0.72 (*p*=0.00023), and mean IMT was decreased from 1.09±0.33 to 1.02±0.31 (*p*=0.065) although the difference was not significant. On the other hand in the control group, no significant change was seen in max IMT (from 1.90±0.55 to 1.99±0.68 (*p*=0.296)), nor mean IMT (from

1.08±0.27 to 1.13±0.34 ( $p=0.217$ )). After exenatide treatment, HbA1c, fasting plasma glucose, and 2-hour-postprandial plasma glucose were significantly decreased from 7.7±1.7 to 6.6±0.4% ( $p=0.0038$ ), 140±31 to 105±15mg/dl ( $p=0.0061$ ), 211±50 to 148±29mg/dl ( $p=0.0011$ ), respectively. Also, serum LDL cholesterol and triglyceride levels were decreased from 120±28 to 105±29mg/dl ( $p=0.0098$ ), 181±125 to 145±79mg/dl ( $p=0.0073$ ), and BMI from 27.7±6.1 to 23.5±9.2 ( $p=0.011$ ), respectively. In the control group, the dose of OHA and/or insulin was increased when necessary, and HbA1c, fasting plasma glucose, and 2-hour-postprandial plasma glucose were significantly decreased from 7.6±1.7 to 7.0±0.4% ( $p=0.0072$ ), 148±34 to 112±17mg/dl ( $p=0.0097$ ), and 189±56 to 162±34mg/dl ( $p=0.043$ ), respectively. No significant change was seen in serum LDL cholesterol and triglyceride levels. BMI tended to increase from 26.8±5.8 to 27.5±4.9 ( $p=0.072$ ).

**Conclusion:** Exenatide therapy resulted in the significant decrease of not only body weight, plasma glucose and serum lipid levels but also in max IMT in patients with type 2 diabetes. Exenatide is suggested to prevent worsening of arteriosclerosis in type 2 diabetes.

## 784

### Incidence of cardiovascular events in patients with type 2 diabetes mellitus treated with DPP-4 inhibitors and sulfonylureas in clinical practice in Germany and the UK: a retrospective analysis

M. Dworak<sup>1</sup>, J.-B. Gruenberger<sup>2</sup>, G. Bader<sup>3</sup>, K. Kostev<sup>3</sup>, W. Rathmann<sup>4</sup>, G. Giani<sup>4</sup>

<sup>1</sup>Novartis Pharma GmbH, Nuernberg, Germany, <sup>2</sup>Novartis Pharma AG, Basel, Switzerland, <sup>3</sup>IMS Health, Epidemiology, Frankfurt, Germany,

<sup>4</sup>Institute of Biometrics and Epidemiology, German Diabetes Center, Duesseldorf, Germany.

**Background and aims:** Antidiabetic treatment should be safe and well-tolerated. Dipeptidyl peptidase-4 (DPP-4) inhibitors and sulfonylureas (SUs) are well established treatments for type 2 diabetes (T2DM). However, previous studies have shown that SUs might be associated with increased mortality and cardiovascular risk. So far no data on the incidence of macro- and microvascular events in patients with T2DM treated with DPP-4 inhibitors or SUs by primary care physicians is available.

**Materials and methods:** Data from patients treated with DPP-4 inhibitors ( $n=12,856$ ) and SUs ( $n=12,856$ ) from general medical practices ( $n=1,201$  physicians) in Germany (Disease Analyser database; 04/2007 to 07/2010) were retrospectively analysed after matching for age ( $67 \pm 11$  years) and sex (males: 58%). Hazard ratios (HR; Cox regression) for macro- and microvascular end points (follow-up: 2 years) were adjusted for type of practice (diabetologist), practice region, health insurance status (private), antidiabetic co-medication, hypertension, hyperlipidaemia, episodes of hypoglycaemia, and the Charlson Comorbidity Index. In UK practices, a similar analysis of patients on DPP-4 inhibitors ( $n=796$ ) and on SUs ( $n=796$ ) was carried out.

**Results:** The risk of macrovascular events was 25% lower in patients treated with DPP-4 inhibitors than in those treated with SUs ( $p<0.001$ ). There was a decreased risk of coronary heart disease (HR: 0.75; 95% CI: 0.67, 0.84), incident stroke/transient ischaemic attacks (HR: 0.56; 95% CI: 0.47, 0.68) and peripheral arterial occlusive disease (HR: 0.73; 95% CI: 0.64, 0.84). There was also a trend toward a decreased risk of myocardial infarction (HR: 0.83; 95% CI: 0.69, 1.01). These results were similar for patients with statutory or private health insurance. A similar trend of macrovascular events was found in the UK, however, not statistically significant due to the small number of cases (HR: 0.77; 95% CI: 0.54, 1.10). No association with microvascular complications was observed. Recorded hypoglycaemias were significantly associated with a higher risk of macrovascular complications (HR: 1.6; 95% CI: 1.1, 2.2).

**Conclusion:** This retrospective database analysis showed that compared to treatment with sulfonylureas, prescription use of DPP-4 inhibitors was associated with a reduced incidence of macrovascular events in patients with T2DM in primary care practices.

Supported by: Novartis Pharma

## 785

### Dipeptidyl peptidase-4 inhibitor (sitagliptin) improves insulin sensitivity and atherosclerosis-related biomarkers in patients with type 2 diabetes

T. Yamane<sup>1</sup>, Y. Suzuki<sup>1</sup>, R. Iwai<sup>2</sup>, Y. Sakuma<sup>2</sup>, S. Yoshida<sup>3</sup>, N. Hashimoto<sup>4</sup>, <sup>1</sup>Diabetes and Metabolic Disease, Asahi General Hospital, Chiba, <sup>2</sup>Clinical Laboratory, Asahi General Hospital, Chiba, Japan, <sup>3</sup>Internal Medicine, Asahi General Hospital, Chiba, <sup>4</sup>Diabetes, Endocrine and Metabolic Disease, Tokyo Women's Medical University, Chiba, Japan.

**Background and aims:** Dipeptidyl peptidase (DPP)-4, an enzyme catalyzing incretins, has been recently reported to be secreted from adipocytes and to be intimately associated with the pathogenesis of obesity and metabolic syndrome. DPP-4 inhibitors therefore may play important roles upon a reduction in insulin resistance and an inhibition of development of atherosclerosis in addition to their glucose lowering effects. The aim of the present study was to examine the effects of sitagliptin, one of DPP-4 inhibitors, upon insulin sensitivity and atherosclerosis in patients with type 2 diabetes.

**Materials and methods:** A total of 131 Japanese patients with type 2 diabetes were enrolled in the study with the informed consent: 73 males/58 females, age  $61 \pm 12$  years, estimated duration of the disease  $11 \pm 7$  years, body mass index  $25.8 \pm 4.7$  kg/m<sup>2</sup>, and HbA<sub>1c</sub>  $7.9 \pm 1.1$  %. They were treated with 50mg of sitagliptin for 24 weeks in addition to the preceding anti-diabetic agents. Laboratory examination included glucose and lipid profiles, indexes of insulin sensitivity (HOMA-IR and quantitative insulin sensitivity check index or QUICKI) and insulin secretion capacity (HOMA- $\beta$  and C-peptide index or CPI). High sensitive C-reactive protein (hsCRP), interleukin (IL)-6, adiponectin, YKL-40 and urinary albumin-to-creatinine ratio (UACR) were assessed as markers for atherosclerosis.

**Results:** Sitagliptin add-on therapy significantly improved parameters for glycaemic control (HbA<sub>1c</sub>:  $7.9 \pm 1.1$  vs.  $7.3 \pm 1.0$  %,  $p<0.0001$ , 1,5-AG:  $8.0 \pm 6.8$  vs.  $9.7 \pm 7.1$  µg/ml,  $p<0.0001$ ) and lipid profiles (LDL-C:  $102 \pm 27$  vs.  $97 \pm 26$ ,  $p<0.01$ , HDL-C:  $52 \pm 11$  vs.  $54 \pm 11$ ,  $p<0.0005$ , FFA:  $575 \pm 243$  vs.  $526 \pm 231$  µEq/l,  $p<0.005$ ). Insulin sensitivity was clearly improved (HOMA-IR:  $3.2 \pm 2.3$ , vs.  $2.7 \pm 2.0$ ,  $p<0.005$ , QUICKI:  $0.336 \pm 0.036$  vs.  $0.344 \pm 0.037$ ,  $p<0.0001$ ), but insulin secretion capacity was unchanged under the fasting conditions. Interestingly serum adiponectin levels were significantly increased ( $8.83 \pm 5.92$  vs.  $9.70 \pm 7.25$  µg/ml,  $p<0.01$ ). In addition, sitagliptin evidently reduced serum levels of inflammatory markers such as hsCRP ( $130 \pm 86$  vs.  $102 \pm 141$  µg/dl,  $p<0.05$ ), IL-6 ( $2.37 \pm 2.12$  vs.  $1.86 \pm 1.07$  pg/ml,  $p<0.01$ ), and YKL-40 ( $116.6 \pm 68.7$  vs.  $105.6 \pm 58.6$  ng/ml,  $p=0.0001$ ). UACR was also decreased ( $47.8 \pm 67.4$  vs.  $39.9 \pm 56.4$  mg/gCre,  $p<0.005$ ).

**Conclusion:** Sitagliptin significantly improved insulin sensitivity and atherosclerosis-related biomarkers associated with the development of inflammation and endothelial dysfunction, suggesting that the agent possesses the action beyond glucose lowering effects. Thus sitagliptin may have some potential roles different from GLP-1 receptor agonists through DPP-4 inhibition.

## 786

### Glucose-dependent insulinotropic polypeptide prevents the progression of macrophage-driven atherosclerosis in diabetic apolipoprotein E-null mice

N. Masaharu, Y. Nogi, M. Terasaki, K. Notomi, M. Tomoyasu, T. Hirano; Department of Medicine, Division of Diabetes, Metabolism, and Endocrinology, Showa University School of Medicine, Shinagawa Tokyo, Japan.

**Background and aims:** We recently reported that glucose-dependent insulinotropic polypeptide (GIP) prevents the development of atherosclerosis in apolipoprotein E-null (Apoe<sup>-/-</sup>) mice. GIP receptors (GIPRs) are found to be severely down-regulated in diabetic animals. We examined whether GIP can exert anti-atherogenic effects in diabetes.

**Materials and methods:** Nondiabetic Apoe<sup>-/-</sup> mice, streptozotocin-induced diabetic Apoe<sup>-/-</sup> mice, and db/db mice were administered GIP (25 nmol/kg/day) or saline (vehicle) through osmotic minipumps for 4 weeks. The animals were assessed for aortic atherosclerosis and for oxidized low-density lipoprotein (ox-LDL)-induced foam cell formation in exudate peritoneal macrophages.

**Results:** Diabetic Apoe<sup>-/-</sup> mice of 21 weeks of age exhibited more advanced atherosclerosis than nondiabetic Apoe<sup>-/-</sup> mice of the same age. GIP infusion in diabetic Apoe<sup>-/-</sup> mice increased plasma total GIP levels by 4-fold without improving plasma insulin, glucose, or lipid profiles. GIP infusion significantly suppressed macrophage-driven atherosclerotic lesions, but this effect was

abolished by co-infusions with [Pro3]GIP, a GIPR antagonist. Ox-LDL-induced cholesterol ester (CE) accumulation in macrophages was 3-fold higher in diabetic Apoe<sup>-/-</sup> mice than in nondiabetic Apoe<sup>-/-</sup> mice. Foam cell formation in macrophages from GIP-infused diabetic Apoe<sup>-/-</sup> mice was suppressed significantly, by 50%. The GIP-induced suppression of foam cell formation was abolished by the co-infusion with [Pro3]GIP. Foam cell formation was 5-fold higher in diabetic db/db mice than in db/mist mice. The GIP infusion attenuated the foam cell formation significantly, by 40%. In vitro infusion of GIP (1 nM) reduced foam cell formation by 15% in macrophages from diabetic Apoe<sup>-/-</sup> mice, and this attenuating effect was weaker than that attained by the same treatment of macrophages from nondiabetic counterparts (35%). While GIPR expression was reduced by only about a half in macrophages from diabetic mice, it was reduced much more dramatically in pancreatic islets from the same animals. Incubation with high glucose (500 mg/dl) for 9–10 days markedly reduced GIPR expression in pancreatic islet cells, but not in macrophages.

**Conclusion:** Chronic administration of GIP remarkably suppressed the progression of atherosclerosis in STZ-induced diabetic Apoe<sup>-/-</sup> mice and suppressed macrophage foam cell formation in both diabetic Apoe<sup>-/-</sup> mice and diabetic db/db mice, even though GIPR expression in macrophages was mildly down-regulated in the diabetic state. This down-regulation of GIPR in macrophages may have been limited enough to permit the suppression of foam cell formation by 4-fold increase of circulating GIP levels.

## PS 061 Oral therapies: metformin, sensitizers and sulfonylureas

### 787

#### Pioglitazone and bladder malignancy during observational follow-up of PROactive: 6-year update

R. Spanheimer<sup>1</sup>, E. Erdmann<sup>2</sup>, E. Song<sup>3</sup>, A.-R. Van Troostenburg de Bruyn<sup>3</sup>, A. Perez<sup>3</sup>;

<sup>1</sup>Takeda Pharmaceuticals North America, Deerfield, USA, <sup>2</sup>Medical Clinic III, University of Cologne, Germany, <sup>3</sup>Takeda Global Research & Development Center, Deerfield, USA.

**Background and aims:** PROactive was a double-blind, placebo-controlled outcomes study investigating pioglitazone for secondary prevention of macrovascular events in type 2 diabetes. Although there was no difference in the cumulative incidence of overall malignancies between treatment groups (3.7% for pioglitazone vs 3.8% for placebo), an imbalance in the number of diagnosed bladder malignancies was reported (n=14 for pioglitazone vs 5 for placebo). Of the 5238 randomized patients, 3599 (74%) then entered a 10-yr non-interventional observational study with no allocation to study medication, which included reporting of any new malignancies.

**Materials and methods:** An interim 6-yr analysis compared overall and bladder malignancies based on original randomization to pioglitazone or placebo.

**Results:** During the observational period alone (mean 5.8 yr), diagnoses of any malignancy were similar between the two groups (Table), and there were fewer cases of bladder malignancy in the pioglitazone group. For the combined PROactive double-blind and observational periods (up to 9.5 yr in total; mean 8.7 yr), a similar % of patients had a diagnosis of any malignancy or specifically bladder malignancy in the pioglitazone and placebo groups (Table). When pioglitazone usage during the observational period was considered, there was no significant difference in bladder malignancies between those with any and those with no pioglitazone use for both periods combined (Hazard ratio=0.98, 95% CI [0.55, 1.77], p=0.96).

**Conclusion:** The imbalance in bladder cancer incidence during the double-blind period of PROactive did not persist in this interim analysis of the observational follow-up study.

Number (%) of patients with a diagnosis of any malignancy or specifically bladder malignancy during PROactive double-blind period and observational follow-up according to original double-blind therapy and regardless of subsequent treatment				
Treatment Period and Endpoint	Original treatment during double-blind period		Relative risk	95% CI
	Pioglitazone	Placebo		
Observational period only	(n=1820)	(n=1779)		
Any malignancy	164 (9.0%)	156 (8.8%)	1.03	[0.83, 1.27]
Bladder malignancy	10 (0.5%)	17 (1.0%)	0.57	[0.26, 1.25]
Double-blind period + observational period	(n=2605)	(n=2633)		
Any malignancy	257 (9.9%)	247 (9.4%)	1.05	[0.89, 1.24]
Bladder malignancy	23 (0.9%)	22 (0.8%)	1.06	[0.59, 1.89]

Supported by: Takeda

### 788

#### Efficacy of sulfonylurea treatment for glycaemic control in diabetes: a systematic review and meta-analysis

A.J. Farmer<sup>1,2</sup>, R.J. Stevens<sup>1,2</sup>, A. Tochlin<sup>3</sup>, T.W.C. Lung<sup>4</sup>, J.A. Hirst<sup>1,2</sup>;

<sup>1</sup>Department of Primary Care Health Sciences, University of Oxford, UK,

<sup>2</sup>National Institute for Health Research School for Primary Care Research, Oxford, UK, <sup>3</sup>Oxford Medical School, University of Oxford, UK, <sup>4</sup>University of Sydney, Sydney School of Public Health, Australia.

**Background and aims:** Sulfonylureas are the oldest class of oral medication for treating diabetes, but the size of treatment on glycated haemoglobin levels (HbA1c) is not well understood. An American Diabetes Association Consensus report from 2009 estimated that sulfonylureas lower HbA1c by 1.5%, based on the results from a single trial. A recent systematic review of 6 trials estimated the reduction in HbA1c to be closer to 1%. We aim to identify randomised controlled trials of sulfonylurea treatment as a monotherapy and added-on to another glucose-lowering medication to examine their effect on HbA1c.

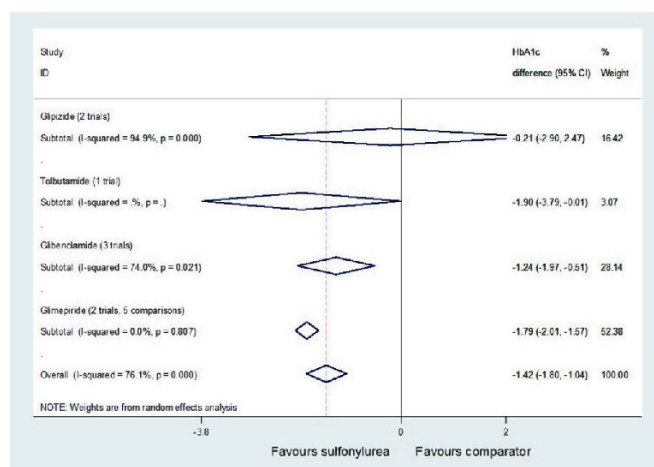
**Materials and methods:** Three databases were searched: Medline, Embase and the Cochrane Library of Registered Controlled Trials. Re-



tried records were screened by 2 reviewers to identify randomised controlled trials of at least 12 weeks duration in which diabetes patients were treated with a fixed dose of sulfonylurea as a monotherapy, added-on to another glucose-lowering medication or compared to a different sulfonylurea dose. HbA1c results were pooled in a random effects meta-analysis. Different sulfonylurea types were examined in subgroup analyses. The effect of trial quality and trial size was investigated in sensitivity analyses.

**Results:** Thirty-two trials were included. In eight trials of sulfonylureas as a monotherapy, HbA1c was on average 1.42% (15 mmol/mol) lower (95% confidence intervals, CI, 1.04–1.80%) with sulfonylurea compared to placebo (Figure). In four trials of sulfonylureas added-on to another oral medication HbA1c was on average 1.62% (18 mmol/mol) CI 1.0 to 2.24%, lower compared to background therapy alone. In seventeen trials of sulfonylurea added-on to insulin treatment HbA1c was on average 0.46% (6 mmol/mol), CI 0.24–0.69% lower than insulin plus placebo. Average insulin dose was 30% lower in the sulfonylurea treated group than the comparator group. Four dose comparison trials did not find any evidence that higher dose sulfonylureas reduce HbA1c more than lower dose sulfonylureas (mean difference 0.05%, CI -0.17 to 0.26). Sensitivity analyses did not show any significant impact of trial quality or size where analyses were possible.

**Conclusion:** Sulfonylureas administered as monotherapy and added-on to oral glucose-lowering medication have been found to lower HbA1c more than a previous systematic review. Sulphonylureas added-on to insulin treatment also lowered HbA1c more than insulin alone and allowed a lower insulin dose. We found no evidence that an increase in sulfonylurea dose increases the reduction in HbA1c. Our results use more trial data than in any previous report and give support to the current recommendations for sulfonylurea treatment.



Supported by: NHS Diabetes

## 789

### HbA<sub>1c</sub> before and after starting treatment with metformin in a large diabetes type 2 cohort

M. Vazquez-Montes, R.J. Stevens, J. Oke, J.K. Aronson, A.J. Farmer;  
Department of Primary Care Health Sciences, University of Oxford, UK.

**Background and aims:** Incentives for improving care in the United Kingdom (UK) Quality Outcomes Framework (QOF) emphasize the use of HbA1c to monitor and titrate treatment of diabetes. Randomised control trials provide evidence that oral glucose reduction treatments lead to improved outcomes in type 2 diabetes. Metformin is the recommended first choice for oral therapy and is the most commonly prescribed glucose lowering agent in the USA and UK. A recent meta-analysis has shown that in metformin monotherapy trials in patients with type 2 diabetes, HbA1c was reduced by 12 mmol/mol. We set out to assess the extent to which initiation of metformin monotherapy in clinical practice is associated with changes in HbA1c levels similar to those observed in trials, by retrospectively analysing routinely recorded primary care data from UK patients with type 2 diabetes.

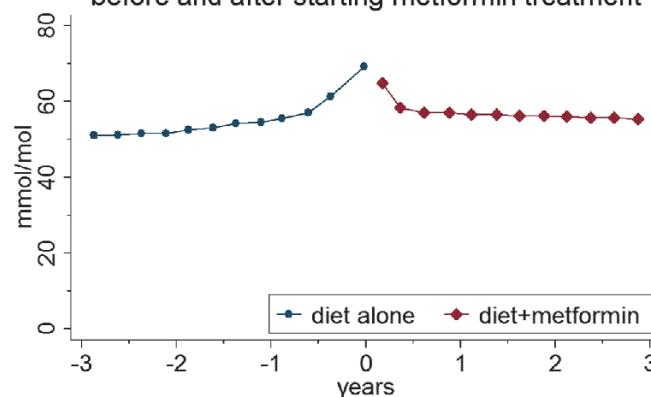
**Materials and methods:** A cohort of 14,677 patients with type 2 diabetes taking no glucose lowering treatments, who started taking metformin monotherapy, was extracted from the General Practice Research Database (GPRD), a computerised database of longitudinal UK medical records. We plotted

HbA1c over time before and after initiation of metformin. We compared the graphical estimate of the change in HbA1c after starting metformin with estimates from an age-adjusted log-linear random effects model that allowed for dosage of metformin and time after starting metformin.

**Results:** Patients included in the cohort had a mean (sd) age of 63 (11) years at baseline and 57% were male. The BMI (sd) was 29.8 (2.0) kg/m<sup>2</sup> at baseline and 29.9 (2.5) kg/m<sup>2</sup> at the start of treatment. HbA1c increased before metformin treatment, fell abruptly at the start of metformin treatment, and fell gradually thereafter (Figure). Mean daily metformin dose was 1066 mg at initiation, and 1162 mg (n = 5419), 1269 mg (n = 4144) and 1334 mg (n = 3256) after 1, 2, and 3 years respectively. A log-linear random-effects model fitted to the same data indicated an average 12% relative HbA1c reduction at the start of metformin therapy (e.g. from 70.0 to 61.6 mmol/mol). Average change in HbA1c varied with dose by 2.1% (relative change) per additional 500 mg/day of metformin. HbA1c increased on average by 3.6% per year (relative change) before starting metformin and 1.8% per year while on metformin, after allowing for the dosage changes over time.

**Conclusion:** HbA1c fell by about 12% after initiation of metformin comparable to the changes previously reported in a meta-analysis of RCTs. The model shows that in individual patients, HbA1c increased at a slower rate after metformin initiation than with diet alone. However, the figure illustrates that in the cohort, HbA1c fell gradually, probably because of dosage changes and because some patients dropped out of this monotherapy cohort.

### HbA1c in patients with type 2 diabetes before and after starting metformin treatment



Supported by: NHS Diabetes

## 790

### Can metformin improve survival in severe metformin-associated lactic acidosis?

F. Kajbaf, J.-D. Lalau;  
Endocrinology-Nutrition, University Hospital, Amiens, France.

**Background and aims:** Analysis of the prognostic values of blood pH and lactate and plasma metformin concentrations in severe metformin-associated lactic acidosis may help to resolve the following paradox: metformin provides impressive, beneficial effects but is also associated with life-threatening adverse effects.

**Materials and methods:** On the basis of 869 pharmacovigilance reports on MALA with available data on arterial pH and lactate concentration, plasma metformin concentration and outcome, we selected cases with a pH < 7.0 and a lactate concentration > 10 mmol/L. The outcomes were compared with those described for severe metformin-independent lactic acidosis by Friesecke et al. (Crit. Care 2010).

**Results:** Fifty-six patients met the above-mentioned criteria. The mean pH and lactate concentration were  $6.75 \pm 0.17$  and  $22.9 \pm 6.9$  mmol/L, respectively. The survival rate was 53%, even with pH values as low as 6.5 and lactate concentrations as high as 35.3 mmol/L. Survivors and non-survivors did not differ significantly in terms of the mean arterial pH and lactate concentration. The mean metformin concentration was higher in patients who subsequently died but this difference was due to a very high value in one patient in this group (188 mg/L, N < 1 mg/L). Sepsis and multidrug overdoses were significantly more frequent in the non-survivors ( $p = 0.017$  and  $0.041$ , respectively). There were no survivors in Friesecke's study of metformin-independent lactic acidosis, despite less severe acidosis on average (mean pH: 6.86).

**Conclusion:** Blood pH and lactate did not have prognostic value in 56 cases of severe metformin-associated lactic acidosis. Strikingly, most patients survived despite a mean pH that is incompatible with favorable outcomes under other circumstances.

## 791

### Low-dose pioglitazone improves glycaemic control, whole-body, and adipocyte insulin resistance, and reduces plasma fractalkine, a novel cytokine in type 2 diabetes

D. Tripathy, G. Daniele, Z. Perez-Cadena, A. Chavez, S. Kamath, F. Andreozzi, T.V. Fiorentino, A. Gastaldelli, R. DeFronzo, F. Folli; Diabetes, Medicine, University of Texas Health Science Center, San Antonio, USA.

**Background and aims:** Pioglitazone (PIO) is a potent insulin-sensitizer but its use is limited by weight gain and fluid retention at higher doses (30–45mg/day). Fractalkine (FRK) is a novel chemokine implicated in the pathogenesis of several inflammatory conditions. The aim of the study was to examine the effect of low dose PIO (15mg/day) on metabolic parameters, body weight, and body composition, and on FRK, and whole body and adipose tissue insulin resistance (IR).

**Materials and methods:** 20 type 2 diabetes (T2D) patients (BMI:  $33.5 \pm 1.3$  Kg/m<sup>2</sup>, FPG:  $145 \pm 8$ , Hb1c:  $7.5 \pm 0.3\%$ ) were randomised to receive PIO (15mg/day, n=11) or placebo (PCB, n=9) for 6 months. All subjects participated in an OGTT with insulin and C-peptide measured every 30 min, euglycemic-hyperinsulinemic clamp (80mU/m<sup>2</sup>-min) with measurement of FFA, adipose tissue IR (AT-IR=FFA  $\times$  insulin) and body composition by DXA at 0 and 6-m. Plasma FRK was measured during OGTTs and insulin clamps and at 0, 1, 3, 5 and 6-m.

**Results:** At baseline, subjects in both groups had similar age, BMI, % body fat (BF), HbA<sub>1c</sub>, fasting, and 2-h plasma glucose. PIO led to a greater reduction in fasting PG ( $140 \pm 11$  to  $118 \pm 9.5$  vs  $150 \pm 13$  to  $140 \pm 12$  mg/dL,  $p=0.004$ ), 2-h PG ( $265 \pm 16$  to  $243 \pm 15$  vs  $285 \pm 12$  to  $293 \pm 10$  mg/dL,  $p=0.02$ ), HbA<sub>1c</sub> ( $7.0 \pm 0.2$  to  $6.5 \pm 0.2$  vs  $8.0 \pm 0.5$  to  $7.7 \pm 0.5\%$ ,  $p=0.02$ ), and triglyceride levels ( $190 \pm 16$  to  $135 \pm 17$  vs  $207 \pm 49$  to  $180 \pm 44$  mg/dL,  $p=0.005$ ). PIO improved whole-body insulin sensitivity (IS) ( $3.2 \pm 0.5$  to  $4.1 \pm 0.5$  vs  $3.4 \pm 0.5$  to  $4.5 \pm 0.5$  mg/kg/min,  $p=0.005$ ) and AT-IR ( $p<0.05$ ). At baseline fasting plasma FRK levels were  $33[38]$  pg/ml (median [IQR range]), similar in PIO vs PCB ( $33[30]$  vs  $29[42]$  pg/ml,  $p=ns$ ). After 6-m, FRK decreased in PIO both during fasting state and clamp ( $13[24]$  and  $19[36]$   $p<0.01$  vs 0 m), whereas in PCB group FRK tended to increase during clamp ( $40[34]$ ;  $p=0.06$  vs 0m,  $p<0.003$  vs PIO). During the entire 6 month period FRK significantly increased in PCB ( $51[69]$  pg/ml, mean of 6-m,  $p<0.007$  vs 0 m) but not in PIO ( $40[32]$  pg/ml,  $p=ns$  vs 0 m,  $p<0.02$  vs PCB). At baseline FRK was associated with AT-IR after correcting for BF ( $r=0.75$ ,  $p=0.002$ ), and after 6 m the decrease in FRK was associated with decrease in AT-IR after correcting for changes in BF and improvement in glycemic control ( $r=0.62$ ,  $p=0.05$ ).

**Conclusion:** In conclusion, (i) low-dose PIO therapy improves glycemic control, lipid profile and whole-body and adipose insulin resistance with minimal weight gain, (ii) plasma FRK is associated with AT-IR, and PIO mediated improvement in AT-IR could partly be due to decreased plasma FRK.

Clinical Trial Registration Number: 01223196

Supported by: Takeda Pharmaceuticals

## 792

### Initial glucose-lowering therapy and cardiovascular outcomes in type 2 diabetes

I.V. Misnikova, A.V. Dreval, Y.A. Kovaleva; Endocrinology, Moscow Regional Research Clinical Institute, Russian Federation.

**Background and aims:** Association of glucose-lowering therapy with cardiovascular risk still is not clear. Aim of the study was to assess association of oral hypoglycemic monotherapy prescribed after onset of type 2 diabetes (T2D) with all-cause and cardiovascular mortality and risk of myocardial infarction (MI) and stroke.

**Materials and methods:** Retrospective open cohort study. 5-year risk of all-cause and cardiovascular mortality, MI and stroke was estimated in patients with T2D diagnosed in 2004 and treated by single agent sulphonylureas (SU) or metformin. Data of Moscow County Register was used. Total of 5956 subjects were included. Risk of all-cause mortality, myocardial infarction and

stroke was assessed by multivariable Cox regression analysis. Groups were adjusted by age, sex, angina pectoris and previous MI.

**Results:** Compared with metformin SU associated with increased risk of all-cause mortality 2,1 [1,3–3,1]; cardiovascular mortality 2,0 [1,2–3,4], MI 2,6 [1,4–4,7], stroke 2,6 [1,4–4,2]. Risk of all-cause mortality was higher in patients treated with glibenclamide 2,3 [1,5–3,5] and gliquidone 2,3 [1,4–3,8]; risk of MI was higher on glibenclamide 4,8 [1,9–11,8] compared with metformin. Risk of stroke was higher in patients in the glibenclamide group 3,4 [1,7–6,7] and gliquidone group 2,6 [1,2–5,6] then patients treated with metformin. Patients treated with gliclazide had a significantly lower risk of all-cause mortality 0,8 [0,6–0,97] and cardiovascular mortality 0,6 [0,5–0,8] in comparison with glibenclamide group.

**Conclusion:** SU associated with increased 5-year mortality and cardiovascular risk compared with metformin in T2D patients. Risk associated with gliclazide was not statistically significant higher compared with metformin.

## 793

### Management of thiazolidinedione induced fluid retention in south Indian patients with type 2 diabetes: effect of spironolactone and amiloride

J. Karalliedde<sup>1</sup>, V. Viswanathan<sup>2</sup>, V. Mohan<sup>3</sup>, P. Tilak<sup>2</sup>, S. Poongothai<sup>3</sup>, S. Agarwal<sup>2</sup>, N. Parthasarathy<sup>3</sup>, G. Subramaniyam<sup>2</sup>, D. Manoharan<sup>2</sup>, C. Chandru<sup>3</sup>, L. Gnudi<sup>1</sup>, G. Viberti<sup>1</sup>;

<sup>1</sup>Diabetes, King's College London, UK, <sup>2</sup>Diabetes, M. V Hospital for Diabetes & Diabetes Research Centre, Chennai, India, <sup>3</sup>Diabetes, Madras Diabetes Research Foundation, Chennai, India.

**Background and aims:** In vitro and animal data suggest that peroxisome proliferator activated receptor  $\gamma$  (PPAR $\gamma$ ) agonists stimulate epithelial sodium channel (ENaC) mediated sodium reabsorption in the distal nephron resulting in plasma volume expansion. The long term management of fluid retention induced by PPAR $\gamma$  agonists such as Pioglitazone or Rosiglitazone (RSG) remains contentious.

**Materials and methods:** We evaluated the effects of Amiloride and Spironolactone, diuretics that act in the distal nephron but affect the ENaC distinctively, on attenuating RSG induced fluid retention in a randomized, placebo controlled study in Chennai, India. We studied 260 T2DM patients who received RSG 4mg bd in addition to background oral agents for 4 weeks. Haematocrit (Hct) was used as a surrogate marker of plasma volume change.

**Results:** Of the 260 patients, 70% (n=180) had evidence of plasma volume expansion defined as an absolute reduction in Hct of  $\geq 1.5\%$  after 4 weeks RSG. These 180 patients (70%M), mean $\pm$ SD, age  $48 \pm 8$  yrs, were randomised to 3 treatments arms; RSG 4mg bd + Spironolactone 50 mg od, RSG 4mg bd + Amiloride 10mg od or RSG 4mg bd + placebo (control group) for 24 weeks. The primary end point was change in Hct at 24 weeks in each diuretic arm versus control group. During the 24 weeks Hct continued to fall significantly in the control group by, mean absolute change (95% CI)  $-1.2(-0.3, -2.2)\%$   $p=0.01$ , and Spironolactone group  $-0.7(-0.1, -1.4)\%$   $p=0.02$ , suggesting continued plasma volume expansion whereas there was no significant change with Amiloride  $[0.0(-0.8, 0.8)\%]$ . After adjustment for baseline values, between group analysis demonstrated that Amiloride was superior to placebo in preventing further Hct fall while Spironolactone was non-significantly different from placebo (Amiloride vs. placebo  $p=0.04$ , Spironolactone vs. placebo  $p=0.41$ ; primary end-point Amiloride was superior to Spironolactone). Haemoglobin fell in placebo and Spironolactone groups as compared to no significant change with Amiloride. HbA<sub>1c</sub> improved similarly in all groups. Blood pressure did not change significantly in all groups.

**Conclusion:** There is a high prevalence of RSG induced fluid retention in South Asian patients with T2DM. In these patients Amiloride, a direct blocker of ENaC, was more effective than Spironolactone preventing protracted/sustained fluid retention

Supported by: GlaxoSmithKline

## 794

**Pioglitazone: effect on pancreatic steatosis and beta cell function**I. Lingvay<sup>1</sup>, M. Poduri<sup>1</sup>, E. Szczepaniak<sup>2</sup>, N.M. Maalouf<sup>3</sup>, L. Harrison<sup>1</sup>, L.S. Szczepaniak<sup>2</sup><sup>1</sup>Internal Medicine/Endocrinology, University of Texas Southwestern Medical Center, Dallas, <sup>2</sup>The Heart Institute, Cedars-Sinai Medical Center, Los Angeles, <sup>3</sup>Internal Medicine, University of Texas Southwestern Medical Center, Dallas, USA.

**Background and aims:** An excessive accumulation of triglycerides (TG) within the muscles and liver leads to insulin resistance while deposition in the pancreas may cause beta-cell dysfunction, a theory known as lipotoxicity. While pioglitazone, a PPAR gamma agonist, is known to increase insulin sensitivity and preserve insulin secretion it is unknown if it does this by decreasing ectopic TGs. This study evaluates the effect of pioglitazone on pancreatic TG content in correlation with insulin secretion and sensitivity.

**Material and methods:** We conducted a prospective, randomized, double-blind, placebo controlled trial to evaluate the effect of treatment with pioglitazone 45 mg daily for 6 months in adults with pancreatic TG content >4%. Eligible volunteers had the following measurements at baseline and 6-month follow-up: HbA<sub>1c</sub>, fasting lipid profile, beta-cell function and insulin sensitivity by frequently sampled intravenous glucose tolerance test (FSIVGTT), liver and pancreatic TG content by magnetic resonance spectroscopy (MRS), subcutaneous and visceral fat mass by MRI, and total body fat/lean mass by densitometry. Pancreatic TG measurement in 4 participants (1 placebo and 3 pioglitazone) had to be excluded due to visceral fat contamination. Data are median+/-interquartile range; between group comparisons were carried out using Wilcoxon t-test.

**Results:** Nineteen volunteers (9 placebo and 10 pioglitazone) completed 6-months of follow-up. Baseline characteristics as well as results are shown in Table 1. Pancreatic and hepatic TG content significantly decreased (p=0.04 and p=0.01, respectively). Insulin sensitivity improved significantly and there was a commensurate decrease in insulin secretion, therefore the disposition index remained unchanged. Total body fat was significantly increased in the pioglitazone treated group.

**Conclusion:** Pioglitazone effectively removes fat from ectopic sites, including the pancreas. In these preliminary results, insulin secretion (adjusted for insulin sensitivity) did not change. Longer intervention and follow-up are in progress to evaluate whether a reduction in pancreatic fat will ultimately translate to improvement of beta-cell function.

**Table 1: Baseline and Follow-up Results**

	Placebo (n=9)		Pioglitazone (n=10)		p-value
	Baseline	6-months	Baseline	6-months	
Age (years)	52 (6)		56 (13.5)		
Ethnicity W/H/B/NA	5/3/0/1		7/2/1/0		
Gender (F:M)	9:0		4:6		
Weight (kg)	99.3 (20.5)	98.6 (24.1)	90.6 (4.9)	89.6 (3.7)	0.95
AI/Rg (μU/ml × min)	417.5 (327.7)	500.8 (266.3)	515.3 (541.6)	258.7 (272.7)	0.008
SI (min <sup>-1</sup> per μU/ml × 10 <sup>3</sup> )	1.911 (0.655)	1.639 (0.480)	1.634 (0.997)	2.943 (1.332)	0.015
DI (AI/Rg × SI)	797.6 (725.8)	637.3 (417.9)	710.7 (920.6)	754.8 (690.4)	0.26
Pancreatic TG content (%)	9.40 (3.11)	12.68 (11.38)	15.92 (11.38)	9.09 (6.69)	0.043
Liver TG content (%)	7.88 (19.39)	5.97 (12.09)	9.05 (13.59)	2.35 (1.69)	0.015
Subcutaneous Fat Area (cm <sup>2</sup> )	371 (158)	305 (196)	151 (65)	146 (109)	0.37
Visceral Fat Area (cm <sup>2</sup> )	247 (104)	253 (104)	254 (64)	250 (49)	0.086
HbA <sub>1c</sub> (%)	5.8 (0.4)	5.8 (0.5)	5.9 (0.4)	5.7 (0.3)	0.035
Triglycerides (mg/dl)	99.0 (120.0)	99.0 (107.0)	123.5 (68.5)	92.5 (21.5)	0.051
Total Body Fat (kg)	42.0 (17.0)	41.4 (20.2)	26.4 (10.5)	28.2 (13.6)	0.086
Total Body Lean Mass (kg)	54.5 (4.1)	51.7 (2.6)	60.1 (12.1)	57.2 (11.8)	0.051
Percent Body Fat (%)	45.0 (11.1)	46.8 (11.3)	31.2 (13.3)	33.2 (15.9)	0.028

All data are median (IQR). Ethnicity: W=White, H=Hispanic, B=Black, NA=Native American). AI/Rg= acute phase insulin release. SI= Insulin Sensitivity. DI= Disposition Index.

Clinical Trial Registration Number: NCT00855010

Supported by: NIH Grants UL1 RR024982 and K23RR024470

## 795

**The relationship between BMI and glycaemic control after monotherapy with metformin XR in Chinese patients with type 2 diabetes mellitus**H. Li<sup>1</sup>, L. Ji<sup>2</sup>, Z. Zhu<sup>3</sup>, J. Newman<sup>4</sup><sup>1</sup>Global Clinical Research, Bristol Myers Squibb, Shanghai, China,<sup>2</sup>Endocrinology and Metabolism, People's Hospital of Peking University, China, <sup>3</sup>Global Clinical Research, Bristol Myers Squibb, Princeton, USA,<sup>4</sup>Research and Development, Bristol Myers Squibb, Melbourne, Australia.

**Background and aims:** Recent global studies report the safety and efficacy of monotherapy with metformin XR in normal-weight T2DM patients; however, this has not been investigated in Chinese T2DM patients.

**Materials and methods:** This open-label, 3-arm, multicenter trial in newly diagnosed Chinese T2DM patients was conducted in 20 sites in China. A total of 371 patients were enrolled into 3 groups according to their baseline BMI (normal weight, 24 kg/m<sup>2</sup> < BMI ≤ 18.5 kg/m<sup>2</sup> BMI, n=125; overweight, 28 kg/m<sup>2</sup> < BMI ≤ 24 kg/m<sup>2</sup>, n=122; obese, BMI ≥ 28 kg/m<sup>2</sup>, n=124) as defined in the 2007 Chinese guidelines. The primary efficacy endpoint of this study was the change from baseline in HbA<sub>1c</sub> after a 16-week oral administration of metformin XR. Secondary endpoints were the change from baseline in fasting plasma glucose (FPG), the relative changes from baseline in fasting lipids (TC, LDL-C, HDL-C, and TG), and changes from baseline in CRP, PAI-1, and adiponectin. Patients were treated with an oral dose of metformin XR for 16 wks. The initial dose was 500 mg/d; up-titrated by increments of 500 mg/wk to 1500 mg/d, unless intolerance or hypoglycaemia occurred. A maximum dose of 2000 mg/d metformin XR was permitted from Weeks 4-16.

**Results:** Metformin XR is safe and efficacious in the treatment of normal weight as well as overweight and obese Chinese patients with newly diagnosed T2DM, achieving a mean reduction HbA<sub>1c</sub> of 1.80%. Differences between the normal weight and obese groups suggest that the effect of BMI on the response to metformin is clinically relevant. Improvement in FPG was also demonstrated, with a mean reduction of 2.097 mmol/L in FPG from baseline to final study visit. Fasting lipid levels improved in all groups (↓TC of 0.195 mmol/L; ↓LDL-C of 0.211 mmol/L, ↑HDL-C of 0.037 mmol/L; ↑adiponectin of 4.865 ng/mL).

**Conclusion:** BMI did not impact on the efficacy of metformin XR in Chinese patients with newly diagnosed type 2 diabetes.

Clinical Trial Registration Number: CV138097

Supported by: BMS

## 796

**Adding glimepiride to insulin+metformin in patients with type 2 diabetes of more than ten years duration-a randomised, double-blind, placebo-controlled cross-over study**

Å. Nybäck-Nakell, P.-E. Lins, U. Adamson, L. Landstedt-Hallin;

Department of Clinical Sciences, Division of Medicine, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** To investigate the effect on glycaemic control by adding glimepiride to ongoing treatment with metformin and insulin in patients with type 2 diabetes with more than 10 years duration.

**Materials and methods:** Patients with a diagnosis of type 2 diabetes for at least 10 years, treated with metformin and insulin, but no sulfonylurea, for at least one year prior to the study were included. Glimepiride 4 mg OD or placebo was added, in a randomized order, to ongoing therapy with metformin and insulin. Each treatment period was three months long with a wash-out of six weeks. All kinds of insulin regimens were allowed and were not changed during the study but the insulin doses were reduced if the patient had a fP-glucose less than 5.0 mmol/l or had symptomatic hypoglycaemia. At the end of each treatment period continuous glucose monitoring (CGMS) was performed during 72 hours to monitor nocturnal glycaemia. HbA<sub>1c</sub>, fasting C-peptide as well as postprandial C-peptide after a standardized breakfast were measured. The primary outcome was HbA<sub>1c</sub>.

**Results:** 43 patients, median age 66 years (46-74), with a diabetes duration of 16 years (10-30), BMI 30 kg/m<sup>2</sup> (25-37) and mean HbA<sub>1c</sub> (Mono-S) 7.1±0.7 % (IFCC 64 mmol/mol) were randomized. At baseline fP-glucose was 8.9±2.7 mmol/l. The fasting C-peptide was 0.69 (0.09-2.10) nmol/l which increased after breakfast to 1.50 (0.30-2.70) nmol/l. The total insulin dose used was 0.50 (range 0.08 to 2.68) U/kg. During the placebo period no change was observed in HbA<sub>1c</sub> while a decrease, from 7.0% to 6.4% (p<0.001), was observed with glimepiride. There was no effect of randomization sequence. The insulin dose had to be reduced in 23 patients by 29 % in median (range 2.1-100%). The ratio between C-peptide/glucose increased significantly with glimepiride, in the fasting state from 0.087 to 0.131 (p<0.001) and postprandially from 0.128 to 0.184 (p<0.001) respectively. A stepwise multiple regression analysis of possible predictive factors for response to glimepiride (age, weight, diabetes duration, postprandial C-peptide/glucose ratio, insulin dose U/kg) revealed that higher age and insulin dose/kg were associated with a smaller decrease in HbA<sub>1c</sub>. Twenty patients (67%) were responders to glimepiride when we defined response as a decrease in HbA<sub>1c</sub> of ≥0.5% or a dose reduction of insulin ≥ 20%. No severe hypoglycaemic event was observed in the study. 22 patients reported hypoglycaemic episodes during daytime, of these 67%



occurred during the glimepiride period. When analysing CGMS recordings between midnight and 6 am, hypoglycaemia was defined as two consecutive measurements <3.1 mmol/l. Time spent at these levels were calculated. Five patients had values <3.1 mmol/l during the end of the placebo period. At the end of glimepiride treatment 16 patients had values <3.1 mmol/l and the median time spent at these levels was 55 min (5–280).

**Conclusion:** Even after more than ten years of known diabetes duration addition of glimepiride to insulin and metformin therapy can be effective in lowering HbA<sub>1c</sub> and/or reducing the need for exogenous insulin.

*Clinical Trial Registration Number:* KCTR20100052

*Supported by:* Sanofi

## PS 062 Clinical studies and GLP-1 agonists

### 797

#### Real-world characteristics of patients with type 2 diabetes initiating insulin glargine plus exenatide or insulin glargine plus liraglutide

A. DiGenio<sup>1</sup>, Z. Ling<sup>1</sup>, K.L. Davis<sup>2</sup>, J.L. Meyers<sup>2</sup>, M.R. Dalal<sup>1</sup>;

<sup>1</sup>Sanofi-Aventis U.S., Bridgewater, <sup>2</sup>RTI Health Solutions, Research Triangle Park, USA.

**Background and aims:** Real-world data on patients initiating basal insulin therapy plus glucagon-like peptide-1 (GLP-1) analogs are limited. This study aimed to document real-world baseline characteristics and clinical endpoints of type 2 diabetes mellitus (T2DM) patients receiving treatment with insulin glargine plus liraglutide (GLA + LIR) or insulin glargine plus exenatide (GLA + EXE).

**Materials and methods:** Using the General Electric Centricity database of electronic medical records (EMR), a retrospective analysis was conducted of T2DM patients ≥ 18 years old who initiated a basal insulin between January 2005 and January 2012 with subsequent or simultaneous initiation of exenatide or liraglutide as add-on GLP-1 analog therapy. Patients had ≥ 6 months EMR activity prior to and ≥ 3 months EMR activity after index date, defined as the first date of combined prescription of both drugs. Primary study endpoints included HbA<sub>1c</sub> levels and body weight and were analyzed descriptively in unmatched treatment groups. Analysis of hypoglycemia rates and gastrointestinal related events and analysis of matched cohorts will be conducted as next steps.

**Results:** At baseline, GLA + LIR patients (N = 149) and GLA + EXE patients (N = 468) had similar age (mean [SD], 57.5 [11.4] and 56.3 [11.6] years). A similar proportion of patients in both groups were male (GLA + LIR: 49.0%, GLA + EXE: 45.9%). GLA + LIR and GLA + EXE patients had similar HbA<sub>1c</sub> levels (8.6% and 8.6%) and body weight (109.7 kg and 110.4 kg) at baseline. The Charlson Comorbidity Index score was 0.9 and 0.8 in GLA + LIR and GLA + EXE patients, respectively. At 6 months and 1 year after the index date, GLA + LIR patients showed a significant reduction in HbA<sub>1c</sub> levels from baseline of −0.66% (*P* < 0.0001) and −0.50% (*P* = 0.005). Body weight in the GLA + LIR group was significantly decreased at 6 months (−2.12 kg, *P* < 0.0001), but not significantly at 1 year after index date (−1.46 kg, *P* = 0.22). GLA + EXE patients also showed significant reductions in HbA<sub>1c</sub> levels and body weight. HbA<sub>1c</sub> levels were −0.50% (*P* < 0.0001) and −0.44% (*P* < 0.0001) lower, while body weight was decreased by −1.98 kg (*P* < 0.0001) and −2.19 kg (*P* < 0.0001) at 6 months and 1 year, respectively.

**Conclusion:** This study demonstrates that, when added to insulin glargine, the GLP-1 agonists exenatide and liraglutide can be associated with reductions in HbA<sub>1c</sub> levels and body weight in T2DM patients in a real-world setting. Matched analysis needs to be considered to explore whether the differences in outcomes between both treatment groups are significant after key confounders are controlled.

Table: Demographic and clinical characteristics of GLA + LIR and GLA + EXE patients

Characteristic	GLA + LIR (n = 149)	GLA + EXE (n = 468)
Mean (sd) age, years	57.5 (11.4)	56.3 (11.6)
Male, n (%)	73 (49.0)	216 (46.0)
Mean (sd) CCI score	0.86 (1.5)	0.81 (1.3)
Mean (sd) baseline HbA <sub>1c</sub> , %	8.61 (1.75)	8.55 (1.74)
Mean (sd) baseline weight, kg	109.7 (25.0)	110.4 (24.2)
Mean (sd) change in HbA <sub>1c</sub> , %		
6 months	−0.66 (1.37)*	−0.50 (1.60)*
1 year	−0.50 (1.60)†	−0.44 (1.70)*
Mean (sd) change in body weight, kg		
6 months	−2.12 (2.50)*	−1.98 (4.70)*
1 year	−1.46 (5.75)	−2.19 (6.78)*

CCI, Charlson Comorbidity Index. \* Within-group *P* < 0.0001, † within-group *P* = 0.005

*Study funding and editorial support provided by Sanofi-aventis U.S.*

## 798

**Safety and tolerability of GLP-1 receptor agonists in patients with type 2 diabetes in comparator-controlled DURATION trials**

L. MacConell, S. Chen, K. Gurney, J. Malloy, H. Zhang, M. Zhou, O. Kolterman;  
Amylin Pharmaceuticals, Inc., San Diego, USA.

**Background and aims:** Injectable GLP-1 receptor agonists (RA) provide a novel mechanism for the treatment of type 2 diabetes mellitus (T2DM). The objective of this post hoc analysis was to characterise and compare the safety and tolerability of exenatide once weekly (EQW) vs exenatide twice daily (EBID) or liraglutide in separate comparator-controlled studies.

**Materials and methods:** Individual patient data were analyzed from the ITT populations of the 30 and 26-week DURATION (DUR)-1 and DUR-5 trials that compared EQW vs EBID (n=545) and DUR-6 trial that compared EQW vs liraglutide (n=911). Study designs and patient characteristics were generally the same across trials with the exception of exclusion of drug-naïve patients in DUR-6. The EQW vs EBID trials were pooled and comparisons between treatments were considered separately. Incidence of treatment-emergent adverse events (AEs), exposure-adjusted incidence, and risk difference and its 95% CI were calculated. Duration and recurrence of AEs over time were analysed for gastrointestinal (GI)-related and injection-site-related AEs.

**Results:** Incidences of serious AEs did not differ between treatments for any comparison (Table). Discontinuations due to AEs were similar between treatments in the EQW vs. EBID comparison and higher with liraglutide compared to EQW. The most frequent AEs in any group were gastrointestinal in nature, which were generally mild and mainly occurred upon initiation of treatment with incidences decreasing over time. Incidences of GI-related AEs at treatment initiation and overall were lower with EQW compared to EBID and with EQW compared to liraglutide. Discontinuations due to GI-related AEs were infrequent in all groups, but occurred more frequently with liraglutide than with EQW. Injection-site-related AEs were highest with EQW compared to all other groups, but were generally mild and abated over time. Only 2 patients discontinued due to injection-site-related AEs (both with EQW, including nodule and pruritus). Overall, the incidence of minor hypoglycaemia was low across all groups and occurred more frequently with concomitant SU use. There was no major hypoglycaemia in any group. Pancreatitis, pancreatic cancer, thyroid cancer, and renal-related AEs were rarely reported and incidence was similar between groups.

**Conclusion:** This post hoc analysis found that the overall safety and tolerability profile of the GLP-1 RAs was similar across treatments, with significantly improved gastrointestinal tolerability observed in patients treated with EQW.

Event, n (%)	EQW vs EBID (Pooled, DUR-1 and -5)			EQW vs LIRA (DUR-6)		
	EQW N=277	EBID N=268	Diff (95% CI)	EQW N=461	LIRA N=450	Diff (95% CI)
Serious AEs	11 (4.0)	10 (3.7)	0.2 (-3.0, 3.5)	13 (2.8)	7 (1.6)	1.3 (-0.6, 3.2)
Discontinuations, all	40 (14.4)	43 (16.0)	-1.6 (-7.8, 4.4)	61 (13.2)	59 (13.1)	0.1 (-4.3, 4.5)
Due to AEs	15 (5.4)	13 (4.9)	0.6 (-3.1, 4.3)	12 (2.6)	24 (5.3)	-2.7 (-5.3, -0.2)
Due to GI-related AE	4 (1.4)	11 (4.1)	-2.7 (-5.4, 0.1)	6 (1.3)	19 (4.2)	-2.9 (-5.0, -0.8)
Due to inj-site-related AE	2 (0.4)	0	0.4 (-0.3, 1.1)	0	0	0
GI-related AEs, all	112 (40.4)	127 (47.4)	-7.0 (-15.0, 1.4)	113 (24.5)	183 (40.7)	-16.2 (-22.2, -10.2)
Nausea	58 (20.9)	63 (24.7)	-14.2 (-21.0, -6.3)	43 (9.3)	93 (20.7)	-11.5 (-15.9, -6.8)
Vomiting	22 (7.9)	38 (14.2)	-8.2 (-11.0, -1.0)	17 (3.7)	48 (10.7)	-7.0 (-10.3, -3.8)
Diarrhea	34 (12.3)	24 (9.0)	3.3 (-1.8, 8.5)	28 (6.1)	59 (13.1)	-7.0 (-10.8, -3.2)
Inj-site-related AEs, all	61 (22.0)	34 (12.7)	9.3 (3.0, 15.6)	72 (15.6)	12 (2.7)	13 (9.3, 16.8)
Inj-site-related AEs, specific*	43 (15.5)	6 (2.2)	13.3 (8.7, 17.9)	23 (5)	4 (0.9)	4.1 (1.9, 6.3)
Hypoglycaemia, with SU	15 (5.4)	15 (5.6)	-0.2 (-4.0, 3.6)	45 (9.8)	36 (8)	1.8 (-1.9, 5.5)
Hypoglycaemia, no SU	2 (1.1)	1 (0.8)	0.6 (-1.3, 2.4)	6 (1.3)	4 (0.9)	0.4 (-0.9, 1.8)

Abbreviations: EQW, exenatide once weekly; EBID, exenatide twice weekly; Diff, difference; LIRA, liraglutide; GI, gastrointestinal; AE, adverse event; inj, injection; DUR, DURATION; SU, sulfonylurea. Treatment differences (Diff [95% CI]) are differences in percentages of patients for comparison of pooled EQW vs pooled EBID or EQW vs liraglutide. Hypoglycaemia includes minor and major events. \*Inj-site-related AEs, specific = injection-site rash, pruritus, erythema, and urticaria.

Clinical trial registration number: NCT00308139, NCT00637273, NCT01029886

## 799

**Efficacy and safety of exenatide once weekly in patients with type 2 diabetes mellitus: a post hoc analysis of pooled data from the DURATION clinical trials**

J. Ruggles, M. Grimm, J. Han, C. Weaver, P. Griffin, C. Schulteis, H. Dong, D. Maggs, J. Malloy;  
Amylin Pharmaceuticals, Inc., San Diego, USA.

**Background and aims:** Exenatide is a glucagon-like peptide-1 receptor agonist that has been demonstrated to improve glycaemic control and reduce body weight with low risk of hypoglycaemia in patients with type 2 diabetes (T2DM). The efficacy and safety of exenatide once weekly (EQW), an ex-

tended-release formulation of exenatide, has been assessed in 6 randomised, comparator-controlled, 24- to 30-wk trials (DURATION-1 through -6). In this post hoc analysis, data from patients treated with EQW in these trials were pooled to assess efficacy and safety across a large population over approximately 6 months.

**Materials and methods:** Data from 1379 intent-to-treat patients treated with EQW were pooled. Mean changes from baseline to endpoint (24-30 wk) in HbA<sub>1c</sub>, fasting blood glucose (FBG), body weight, and cardiovascular (CV) risk factors (blood pressure [BP] and fasting lipids) were assessed. Changes in HbA<sub>1c</sub> were also analysed by baseline HbA<sub>1c</sub> (<9% or ≥9%). The percentage of patients achieving HbA<sub>1c</sub> goals without weight gain and hypoglycaemia was determined.

**Results:** At baseline, ITT patients were 55% male, with a mean±SD age of 55±10 y, HbA<sub>1c</sub> of 8.4±1.1%, BMI of 32.5±5.4 kg/m<sup>2</sup>, FBG of 9.6±2.7 mmol/L, and duration of T2DM of 7±6 y. Improvements in glycaemic control were observed following 24-30 wk of treatment with EQW (LS mean change from baseline±SE: HbA<sub>1c</sub>, 1.4±0.0%; FBG, 2.0±0.1 mmol/L), with significant reductions in HbA<sub>1c</sub> evident by week 4 of treatment. Patients with baseline HbA<sub>1c</sub> <9% achieved an LS mean reduction±SE of -1.1±0.0%, whereas patients with baseline HbA<sub>1c</sub> ≥9% achieved a larger reduction of 2.2±0.1%. At endpoint, 59% of patients achieved HbA<sub>1c</sub> <7%, and 39% achieved HbA<sub>1c</sub> ≤6.5%. EQW treatment resulted in significant weight loss at endpoint (LS mean change±SE: 2.5±0.1 kg), with 76% of patients losing weight; 70% had reductions in both weight and HbA<sub>1c</sub>. Modest but statistically significant improvements were observed in CV risk factors (LS mean change±SE: systolic BP, 2.8±0.4 mmHg; diastolic BP, 0.8±0.2 mmHg; total cholesterol, 0.17±0.02 mmol/L; LDL, 0.10±0.02 mmol/L; triglycerides [geometric LS mean % change±SE, median], 6±1%, 0.09 mmol/L). EQW was generally well tolerated, with a low incidence of adverse events (AEs) leading to withdrawal (4%) and serious AEs (3%). The most common AEs were nausea (16%), diarrhoea (11%), nasopharyngitis (8%), headache (8%), injection-site nodule (7%), vomiting (6%), constipation (6%), and injection-site pruritus (5%). New events of nausea and vomiting decreased over time, and seldom led to withdrawal (0.4% and 0.1%, respectively). No major hypoglycaemia occurred; minor hypoglycaemia occurred primarily in patients using a sulphonylurea (SU; 16%) and occurred in few patients not using an SU (3%). Overall, 45% of patients achieved a clinically relevant composite goal at endpoint of HbA<sub>1c</sub> <7% with no weight gain and no hypoglycaemia, and 31% achieved this goal with HbA<sub>1c</sub> ≤6.5%.

**Conclusion:** Results from this post hoc analysis of >1300 patients demonstrated that exenatide administered once weekly over 24-30 wk provided improvements in glycaemic control, weight loss, and CV risk factors, with low risk of hypoglycaemia. Consistent with the known safety profile, EQW therapy was generally well tolerated, with gastrointestinal AEs occurring most frequently and seldom leading to treatment discontinuation.

Clinical Trial Registration Number: NCT00308139, NCT00637273, NCT00641056, NCT00676338, NCT00877890, NCT01029886

## 800

**Is insulin the most effective injectable HbA<sub>1c</sub>-lowering therapy?**

M. Diamant<sup>1</sup>, A. Peters<sup>2</sup>, D. Russell-Jones<sup>3</sup>, S. Furber<sup>4</sup>, M. Donsmark<sup>4</sup>, J. Han<sup>5</sup>, L. MacConell<sup>1</sup>, D. Maggs<sup>5</sup>, J. Buse<sup>6</sup>;

<sup>1</sup>Endocrinology / Diabetes Centre, VU University Medical Centre, Amsterdam, Netherlands, <sup>2</sup>University of Southern California, Los Angeles, USA, <sup>3</sup>Royal Surrey County Hospital, Guildford, UK, <sup>4</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>5</sup>Amylin Pharmaceuticals, Inc, San Diego, USA, <sup>6</sup>University of North Carolina School of Medicine, Chapel Hill, USA.

**Background and aims:** In recent deliberations, it was concluded that only insulin was deserving of a 'highest' designation in efficacy of HbA<sub>1c</sub> reduction among antihyperglycaemic agents. Basal insulins have been traditionally used to treat patients with serious hyperglycaemia. However, recent data on the GLP-1 receptor agonists (GLP-1RA) exenatide once weekly (ExOW) and liraglutide (LIRA) once daily (QD) suggested superior efficacy to insulin glargine (GLAR).

**Materials and methods:** To assess the effectiveness of GLP-1RA and basal insulin in patients with varying degrees of hyperglycaemia, we conducted analyses of the LEAD-5 and DURATION-3 data to examine HbA<sub>1c</sub>, fasting plasma glucose (FPG), weight and insulin dose by quartiles (Q) of baseline HbA<sub>1c</sub>. These were open-label comparisons respectively of LIRA 1.8 mg QD and ExOW 2 mg versus GLAR QD titrated to FPG <5.5 mmol/L for 26 weeks.

**Results:** The table presents mean values of HbA<sub>1c</sub> for Q1-Q4 at baseline and the change at 26 week for LIRA and ExOW versus GLAR. The change in

HbA<sub>1c</sub> was equivalent or greater at all quartiles of HbA<sub>1c</sub> for both ExOW and LIRA compared to GLAR. Further analyses will be presented on FPG and insulin dose/titration suggesting reasonable insulin titration as well as on change in body weight suggesting additional extra-glycaemic benefits of long-acting GLP-1RA.

**Conclusion:** Clearly, these data demonstrated that in a clinical trial setting, where insulin use can be optimised, GLP-1RA were similar, if not superior in efficacy to basal insulin for HbA<sub>1c</sub> reduction for patients with type 2 diabetes failing 1–2 oral antihyperglycaemic agents.

Study and Demographics	Treatment (n)		Q1	Q2	Q3	Q4
			Mean (SD)			
<b>LEAD-5</b> (add on to MET+SU [100%]) Previous therapy: Monotherapy 6% Dual therapy 94% Age ~58 years DM Duration ~9 years BMI ~31 kg/m <sup>2</sup>	LIRA 1.8 mg (230)	Baseline	7.2 (0.3)	7.9 (0.2)	8.5 (0.2)	9.5 (0.5)
		Change	-0.9 (0.6)	-1.1 (0.8)	-1.4 (0.8)	-1.8 (1.1)
	GLAR (232)	Baseline	7.1 (0.5)	7.9 (0.2)	8.6 (0.2)	9.4 (0.5)
		Change	-0.5 (0.6)	-0.9 (0.7)	-1.2 (0.8)	-1.5 (1.0)
<b>DURATION-3</b> (add on to MET [70%] or MET+SU [30%]) Previous therapy: Monotherapy 70% Dual therapy 30% Age ~58 years DM Duration ~8 years BMI ~32 kg/m <sup>2</sup>	ExOW 2 mg (233)	Baseline	7.1 (0.2)	7.7 (0.2)	8.4 (0.3)	9.9 (0.6)
		Change	-0.8 (0.6)	-1.2 (0.5)	-1.3 (0.9)	-2.3 (1.2)
	GLAR (223)	Baseline	7.1 (0.3)	7.8 (0.2)	8.5 (0.3)	9.8 (0.6)
		Change	-0.6 (0.5)	-0.9 (0.7)	-1.3 (0.8)	-2.1 (0.9)

Supported by: Amylin & Novo Nordisk

## 801

### Liraglutide is effective across a range of obese body mass indices; findings from the Association of British Clinical Diabetologists (ABCD) nationwide liraglutide audit

R.E.J. Ryder, P. Sen-Gupta, K.Y. Thong, ABCD nationwide liraglutide audit contributors;  
City Hospital, Birmingham, UK.

**Introduction:** It is uncertain whether GLP-1 receptor agonist treatment is effective across a range of body mass indices (BMI). We assessed the treatment response to liraglutide stratified according to patients' pre-treatment BMI.

**Methods:** Data was obtained from the ABCD nationwide liraglutide audit. The audit collected anonymised, clinical data of patients routinely treated with liraglutide in clinical practice. Diabetes centres across UK were invited to participate. Data on reduction of HbA<sub>1c</sub>, weight, and weight as a percentage of initial body weight was assessed at three months (± 6 weeks) of liraglutide treatment. These results were compared among patients divided according to six BMI groups of 25–29.9 kg/m<sup>2</sup>, 30–34.9 kg/m<sup>2</sup>, 35–39.9 kg/m<sup>2</sup>, 40–44.9 kg/m<sup>2</sup>, 45–49.9 kg/m<sup>2</sup> and 50–54.9 kg/m<sup>2</sup>. We excluded patients who switched from exenatide to liraglutide therapy, who used liraglutide 1.8 mg rather than the 1.2 mg dose, who used insulin, who had no BMI data, who had BMI <25 kg/m<sup>2</sup> or >55 kg/m<sup>2</sup>, whose follow up data was outside the specified time frame, or who were lost to follow-up.

**Results:** 77 centres contributed data on 4129 patients with mean (SD) HbA<sub>1c</sub> of 9.4% (1.7) and BMI of 38.8 kg/m<sup>2</sup> (7.3). After exclusions, there were 634 and 588 HbA<sub>1c</sub> and weight data for comparisons among the six BMI groups. No difference in HbA<sub>1c</sub> reduction was seen between BMI groups either before (p=0.929) or after adjustment for factors such as baseline HbA<sub>1c</sub> and ethnicity (p=0.894) (Table). Mean HbA<sub>1c</sub> reductions were between -1.1% to -1.4%. In contrast, weight loss increased with greater BMI (p=0.010), ranging from -1.8 kg to -4.4 kg. However, weight loss as a percentage of initial body weight was similar across BMI groups (ranging from -2.2% to -3.1% initial body weight).

**Conclusion:** The use of liraglutide in the UK as reported in the nationwide audit was common among patients with significant obesity. This is likely due to national guidelines that only support the use of GLP-1 receptor agonists in heavier patients. Liraglutide was effective across a range of obese body mass indices among non-insulin-treated patients with type 2 diabetes. Table: HbA<sub>1c</sub> and weight reduction at three months after liraglutide 1.2 mg treatment across patients with BMI 25 to 55 kg/m<sup>2</sup>.

	BMI groups (kg/m <sup>2</sup> )						Overall p-value
	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–54.9	
	(n=37)	(n=161)	(n=204)	(n=123)	(n=76)	(n=33)	
HbA <sub>1c</sub> reduction (%) (Non-adjusted)	-1.4(0.2)	-1.3(0.1)	-1.4(0.1)	-1.3(0.1)	-1.3(0.2)	-1.2(0.3)	0.929
HbA <sub>1c</sub> reduction (%) (Adjusted)	-1.4(0.2)	-1.3(0.1)	-1.3(0.1)	-1.4(0.1)	-1.3(0.1)	-1.1(0.2)	0.894
	(n=34)	(n=153)	(n=182)	(n=119)	(n=71)	(n=29)	
Weight reduction (kg)	-1.8(0.4)	-2.2(0.3)	-2.8(0.3)	-3.4(0.5)	-3.9(0.6)	-4.4(0.8)	0.010
% Body weight reduction	-2.2(0.5)	-2.3(0.3)	-2.6(0.3)	-2.9(0.4)	-3.0(0.5)	-3.1(0.6)	0.649

HbA<sub>1c</sub>, weight and percentage body weight reduction are shown as mean (SE). Mean HbA<sub>1c</sub> was adjusted for baseline HbA<sub>1c</sub>, ethnicity, gender, age and number of oral antidiabetes drugs.

Supported by: Novo Nordisk

## 802

### Liraglutide and metformin combination therapy: clinical benefits associated with early use of liraglutide in type 2 diabetes and with switching a sulphonylurea to liraglutide

S.C. Bain<sup>1</sup>, J. Seufert<sup>2</sup>, A.B. Thomsen<sup>3</sup>, S. Furber<sup>3</sup>, D. D'Alessio<sup>4</sup>;

<sup>1</sup>Department of Medicine, Abertawe Bro Morgannwg University NHS Trust, Swansea, UK, <sup>2</sup>University Hospital of Freiburg, Germany, <sup>3</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>4</sup>University of Cincinnati, USA.

**Background and aims:** When first-line metformin (met) becomes insufficient in type 2 diabetes, there is no general consensus on how to advance treatment. The aim of this post-hoc analysis was to compare the clinical benefits associated with add-on liraglutide 1.8 mg treatment in patients previously receiving met monotherapy (met-add-on group) vs substitution of a sulphonylurea (SU) with liraglutide 1.8 mg in patients previously receiving met + SU combination therapy (SU-switch group).

**Materials and methods:** Data were obtained from a large clinical trial (n=988) in which patients receiving met monotherapy or met + SU (SU dose at ≤ half of the maximum approved dose) had their therapy changed to met + liraglutide 1.8 mg. Liraglutide was initiated at 0.6 mg/day and incremented by 0.6 mg/day on a weekly basis to the final 1.8 mg/day dose.

**Results:** Duration of diabetes was significantly longer in the SU-switch patients compared with the met-add-on group (Table). Among patients who completed 12 weeks of treatment with met + liraglutide 1.8 mg, the SU-switch group lost more weight compared with the met-add-on group. Completers in the met-add-on group had a greater reduction in HbA<sub>1c</sub> compared with the SU-switch group.

**Conclusion:** The greater reduction in weight in the SU-switch group vs the met-add-on group is likely due to the termination of SU treatment. Reductions in HbA<sub>1c</sub> may be positively influenced by the slightly higher baseline HbA<sub>1c</sub> in the met-add-on group and negatively influenced by termination of SU in the switch group, although the latter is a reflection of clinical practice. While liraglutide improved glycaemic control in both subgroups, these data suggest that the effect of liraglutide may depend on the duration of diabetes and prior treatments. These factors should be considered when making treatment decisions and designing trials with liraglutide.



	Met-add-on group (n=532)		Met-SU-switch group (n=285)	
	Baseline	Change from baseline	Baseline	Change from baseline
Age (years)	56 (9.8)	-	58 (9.3)	-
Diabetes duration (years)	6.5 (5.4)**	-	9.0 (6.2)	-
HbA <sub>1c</sub> (%)	8.0 (0.86)	-1.3 (0.04)**	7.7 (0.48)	-0.6 (0.04)
Patients reaching HbA <sub>1c</sub> <7.0% at 12 weeks (%)		69.7	-	44.6
Body weight (kg)	99.4 (21.44)	-3.7 (0.18)*	98.4 (20.03)	-4.4 (0.21)
Fasting plasma glucose (mmol/L)	9.8 (2.24)	-2.2 (0.09)	9.3 (1.84)	-0.8 (0.12)
Systolic blood pressure (mmHg)	134.2 (16.09)	-4.2 (0.79) <sup>NS</sup>	135.1 (15.65)	-3.7 (0.91)

Data are for patients completing 12 weeks' treatment. Baseline data: mean (SD); change data: mean (SE) with no imputation for missing values. NS, \* $p=0.019$  and \*\* $p<0.0001$  vs Met-SU-switch group based on ANCOVA.

Clinical Trial Registration Number: NCT00856986  
Supported by: Novo Nordisk

## 803

### Does liraglutide confer greater glycaemic benefits than exenatide when used with oral agents versus insulin in clinical practice?

Q. Zhang<sup>1</sup>, P. Levin<sup>2</sup>, E. Chou<sup>1</sup>

<sup>1</sup>R&D, Sanofi, Bridgewater, <sup>2</sup>Model Clinical Research, Baltimore, USA.

**Background and aims:** In randomized trials, adding LIRA (liraglutide) versus EXEN (exenatide) to oral agents (OADs) result in greater improvements in A1C and fasting plasma glucose but not in prandial glucose excursions. This study evaluated real world glycemic effects of LIRA vs EXEN when adding to oral agents or insulin.

**Materials and methods:** Patients with A1C  $\geq 7\%$  were identified from IM-PACT™ claims database (Jan 2009-Dec 2010) and evaluated according to the intention to treat A1C response, defined as a  $\geq 0.8\%$  reduction plus any A1C value  $\leq 7\%$  within 6 months after initiation of LIRA or EXEN.

**Results:** The mean age of patients was 52 years for both LIRA (n=878) and EXEN (n=1,638). Previous use of OAD only was 66 vs 71% and majority of insulin therapy was basal, 21 vs 18% in LIRA vs EXEN respectively. Baseline A1C (8.7% vs 8.9%) and comorbidity burden (Charlson comorbidity score: 1.68 vs 1.85) were comparable in LIRA vs EXEN. Six-month A1C response rate was 26% in Lira and 15% in EXEN ( $P<0.01$ ), with a hazard ratio (HR) of 1.76 (95% CI: 1.47-2.11) after adjustment of baseline A1C, concomitant medications, comorbidity, and other baseline characteristics. GLP-1 medication adherence was also used as a covariate and strongly associated with A1C response (HR=3.58; 2.54-5.04). Propensity matched (1:1) response estimates remained unchanged with 26% in LIRA vs 16% in EXEN (Odds Ratio=1.82; 1.42-2.33). Stratified by OAD vs basal insulin therapy, response rate was 28 vs 17% in OAD only (OR=1.95; 1.45-2.61), and 13 vs 14% in basal insulin (OR=0.89; 0.46-1.73) for LIRA vs EXEN.

**Conclusion:** A1C benefit with LIRA shown in clinical trials is confined only to patients on OADs in this real world database study. When adding to insulin, the A1C response rate in LIRA declined more than 50% relative to that in the combination with OADs, showing no difference from the response rate in EXEN. This suggests the clinical need for an alternative GLP-1 therapy with a complementary mode of glucose lowering action to basal insulin to optimize glycemic control.

Supported by: Sanofi

## 804

### Addition of fixed-dose exenatide to insulin glargine therapy improved glycaemic control without increasing hypoglycaemia or weight gain across a range of insulin titration

M. Wintle<sup>1</sup>, R. Penczek<sup>1</sup>, J. Han<sup>1</sup>, S. Miller<sup>1</sup>, J. Buse<sup>2</sup>

<sup>1</sup>Amylin Pharmaceuticals, Inc., San Diego, <sup>2</sup>University of North Carolina, Chapel Hill, USA.

**Background and aims:** In a recent 30-week, randomised, controlled, phase 3 study in patients with type 2 diabetes, the addition of fixed-dose, mealtime exenatide to titrated insulin glargine significantly improved glycaemic control without additional hypoglycaemia or weight gain when compared to titrated insulin glargine and placebo. To better understand the interaction between fixed exenatide dosing and insulin titration, a post hoc analysis was performed to examine these effects in relation to the variable degree of insulin titration that actually occurred as a result of the insulin titration algorithm specified in the protocol.

**Materials and methods:** Study subjects (age  $59\pm 9$  y, HbA<sub>1c</sub>  $8.4\pm 0.9\%$ , weight  $94\pm 21$  kg, duration of diabetes  $12\pm 7$  y, mean $\pm$ SD) on pre-existing ( $\geq 3$  mo) insulin glargine were randomized to placebo (n=123) or exenatide twice daily (n=138; 5 mcg for 4 weeks then 10 mcg for the remainder of the study). Insulin glargine was titrated according to the Treat-to-Target algorithm with the goal of achieving fasting plasma glucose (FPG)  $<5.6$  mmol/L (100 mg/dL). Subjects from both treatments were pooled and divided into 3 tertiles (T1, T2, T3) according to the change in insulin dose between baseline and endpoint. Treatments were compared for effects on FPG, HbA<sub>1c</sub>, weight, and incidence of hypoglycaemia within each insulin titration tertile.

**Results:** Mean insulin titration in each tertile ranged from modest reductions in T1 to substantial increases in T3 (Table). Greater improvements in HbA<sub>1c</sub> were demonstrated in all titration tertiles with the addition of exenatide to insulin glargine compared to placebo plus insulin glargine. Between-treatment differences were statistically significant in T2 and T3 for HbA<sub>1c</sub> reduction (Table) and percentage of subjects achieving HbA<sub>1c</sub>  $<7\%$  (exenatide vs placebo: T1, 44% vs 29%; T2, 65% vs 26%; T3, 54% vs 29%). Percentage of subjects reaching HbA<sub>1c</sub>  $\leq 6.5\%$  was significantly greater with exenatide in all 3 tertiles (T1, 33% vs 9%; T2, 52% vs 10%; T3, 36% vs 16%). Incidence of hypoglycaemia was lower with exenatide in each tertile of insulin titration (Table). Adjunctive exenatide was associated with statistically significant weight loss in T1 and T2 and exenatide significantly inhibited the weight gain associated with the insulin increase in T3 ( $-0.09$  kg vs  $1.96$  kg). A linear regression analysis of insulin titration vs weight change by treatment revealed that, on average, weight gain occurred with the addition of  $\geq 5$  U of insulin in placebo-treated subjects. In contrast, subjects receiving adjunctive exenatide could add up to 33 U without experiencing weight gain or additional hypoglycaemia.

**Conclusion:** The addition of fixed-dose, mealtime exenatide to systematically titrated insulin glargine significantly improved glucose control without increasing the risk of hypoglycaemia and with mitigation of insulin-induced weight gain.

		T1		T2		T3		
		ITT (n)	Exenatide (n=52)	Placebo (n=34)	Exenatide (n=46)	Placebo (n=39)	Exenatide (n=39)	Placebo (n=49)
Insulin dose (U)	BL	52 (31)	49 (25)	42 (17)	40 (19)	55 (39)	51 (29)	
	Δ	-6.7 (1.1)	-5.5 (1.4)	11.1 (0.7)	10.5 (0.7)	35.2 (2.6)	41.0 (2.3)	
FPG (mmol/L)	BL	6.61 (2.34)	6.31 (2.42)	6.99 (2.21)	6.79 (2.07)	8.61 (2.85)	8.82 (2.72)	
	Δ	0.27 (0.38)	-0.12 (0.49)	-0.95 (0.29)	-0.85 (0.32)	-2.26 (0.51)	-1.80 (0.47)	
HbA1c (%)	BL	8.2 (0.8)	8.0 (0.9)	8.2 (0.8)	8.6 (0.8)	8.7 (0.9)	8.8 (1.0)	
	Δ	-1.18 (0.14)	-0.78 (0.18)	-1.65 (0.12)**	-0.85 (0.13)	-1.65 (0.16)*	-1.12 (0.14)	
Weight (kg)	BL	93 (20)	90 (20)	92 (19)	87 (20)	102 (22)	101 (21)	
	Δ	-2.86 (0.48)	-0.12 (0.59)	-1.64 (0.58)	0.02 (0.62)	-0.09 (0.60)*	1.96 (0.54)	
Hypo- glycaemia incidence	%	26.9	32.4	26.1	35.9	20.5	22.4	

BL, baseline. BL values are mean (SD). Change values are LS mean (SEM). \* $P<0.05$ , \*\* $P<0.01$  vs placebo. ANCOVA model with baseline values of the dependent variable being adjusted as a covariate.

Clinical Trial Registration Number: NCT00765817

## 805

# Liraglutide in paediatric subjects with type 2 diabetes: a population pharmacokinetic (PK) analysis and comparison to adult subjects

K.C. Petri<sup>1</sup>, L. Jacobsen<sup>1</sup>, S. Ingwersen<sup>1</sup>, D. Klein<sup>2</sup>;

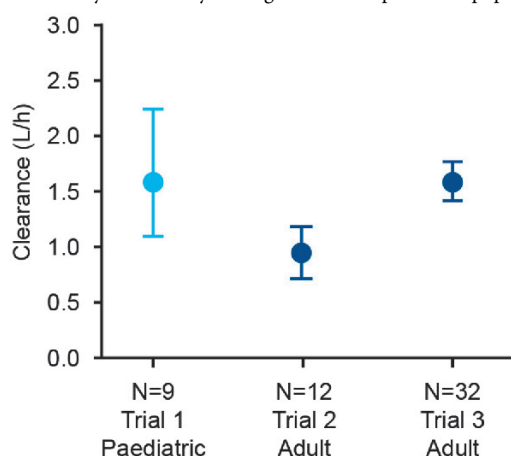
<sup>1</sup>Novo Nordisk, Søborg, Denmark, <sup>2</sup>Cincinnati Children's Hospital Medical Center, Cincinnati, USA.

**Background and aims:** The prevalence of type 2 diabetes (T2D) in the paediatric population is increasing. Only metformin and insulin are approved for the treatment of paediatric patients. Thus, there is a medical need for more treatment options. This analysis investigated the population PK of the once-daily human GLP-1 analogue liraglutide in paediatric subjects (10–17 yrs) with T2D and compared the results to those seen in previous liraglutide PK trials in adults.

**Materials and methods:** A 1-compartment model with 1<sup>st</sup>-order absorption and elimination was used to estimate clearance (CL/F) and volume of distribution (V/F) for each liraglutide dose level in a pooled data set of 3 trials (1 in children, 2 in adults). AUC<sub>0–24h</sub><sup>ss</sup> derived from CL/F and dose was used to investigate dose proportionality in the paediatric trial. A covariate analysis investigated the effects of dose, body weight, gender and age category (paediatric/adult) on liraglutide exposure. Data sources: Demographics and PK data from 3 trials including subjects with T2D. Trial 1: 13 paediatric subjects (8 female), 10–17 yrs with baseline body weight 57–214 kg; frequently sampled plasma samples (0–13 h, 0–24 h for final dose) were collected following once daily liraglutide doses (with sequential weekly increases) of 0.3, 0.6, 1.2, and 1.8 mg. Trial 2: 12 adults (6 female) with body weight 72–104 kg. Trial 3: 32 adults (9 female) with body weight 58–140 kg. Frequently sampled plasma samples (0–60 h, 0–24 h, respectively) from Trials 2 and 3 were collected at 1.8 mg steady state.

**Results:** PK data indicated dose proportionality for the model-derived AUC<sub>0–24h</sub><sup>ss</sup> in the dose range 0.3–1.8 mg in the paediatric subjects with T2D (slope: 1.05 (95%CI 0.96–1.15)). Consistent with findings with sparse PK data from adult phase 3 trials, body weight and gender were significant covariates for liraglutide exposure in the paediatric population. AUC<sub>0–24h</sub><sup>ss</sup> decreased with increasing body weight and was lower for males than for females. Geometric mean AUC<sub>0–24h</sub><sup>ss</sup> was 63% (44–84% CI) higher at the lowest observed body weight (53 kg) and 56% (46–64% CI) lower at the highest observed body weight (216 kg) compared to the exposure at the median body weight of 90 kg. Males had 31% (17–42%) lower exposure (AUC<sub>0–24h</sub><sup>ss</sup>) than females. Exposure in paediatric subjects was not significantly different from adults. The CL/F estimates (Fig), and thus exposure, for the paediatric subjects with T2D were similar to those in the adult trials. The differences between the adult Trials 2 and 3 were partly due to differences in body weight and gender distribution.

**Conclusion:** Based on this population PK analysis, the same liraglutide dose regimen as used in adults, is predicted to achieve the same range of exposures in the paediatric population (10–17 yrs, 57–214 kg) as found clinically effective in adults. A larger scale trial is planned to further investigate the safety, tolerability and efficacy of liraglutide in the paediatric population with T2D.



Geometric mean (95% CI) of post hoc estimated clearance (CL/F) for liraglutide 1.8 mg in Trial 1 (paediatric T2D subjects) and Trials 2 and 3 (adult T2D subjects).

Clinical Trial Registration Number: EudraCT: 2010-021057-39

Supported by: Novo Nordisk

## 806

# Identifying predictors of response to liraglutide in type 2 diabetes using recursive partitioning analysis

R. Ratner<sup>1</sup>, J. Brett<sup>2</sup>, N. Khutoryansky<sup>2</sup>, V.R. Aroda<sup>1</sup>;

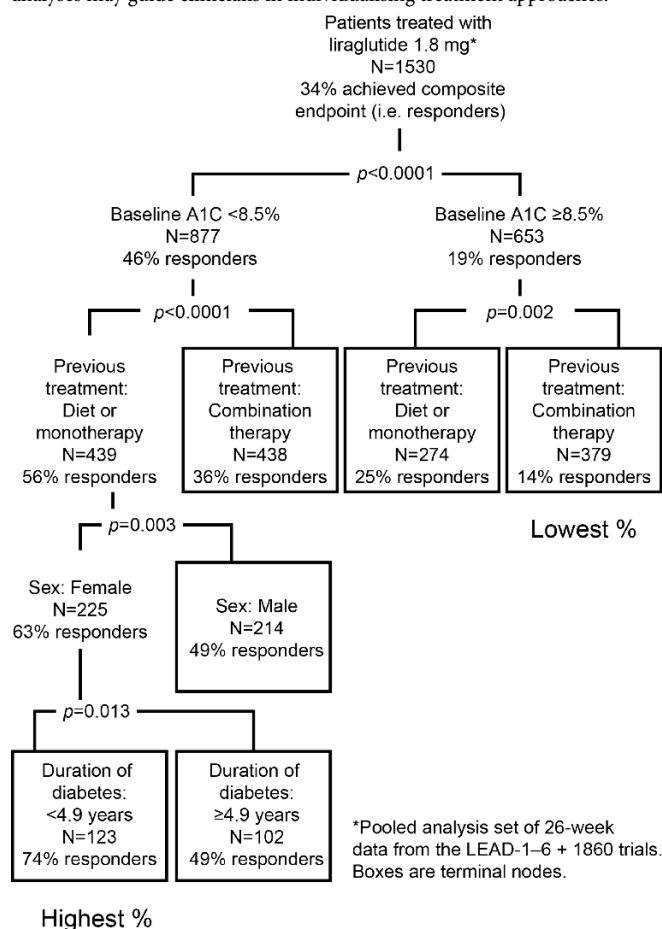
<sup>1</sup>MedStar Health Research Institute, Hyattsville, <sup>2</sup>Novo Nordisk Inc, Princeton, USA.

**Background and aims:** Randomised clinical trials provide unbiased databases for comparative effectiveness analyses to see which patients respond best to available interventions.

**Materials and methods:** We evaluated patient-level data pooled from seven Phase 3 clinical trials with liraglutide to examine responder subgroups, as defined by those achieving a composite endpoint of HbA<sub>1c</sub> <7%, no weight gain and no hypoglycaemia (episodes requiring assistance or self-treated with PG <56 mg/dL [3.1<mmol/L]) over 26 weeks.

**Results:** Overall 34% of individuals on liraglutide 1.8 mg achieved the pre-specified composite endpoint. Candidate predictor variables included baseline age, sex, ethnicity, BMI, HbA<sub>1c</sub>, beta-cell function, FPG, insulin resistance, previous treatments, and diabetes duration. Using recursive partitioning to create classification trees, baseline HbA<sub>1c</sub> was the most significant predictor, with a probability of achieving the composite outcome of 46% when baseline HbA<sub>1c</sub> <8.5%, as opposed to 19% if baseline HbA<sub>1c</sub> ≥8.5% ( $p<0.0001$ ). Subsequent splits (with  $p$ -values <0.05) produced a subgroup within patients with a baseline HbA<sub>1c</sub> <8.5% that was identified by previous treatment with diet or monotherapy, female sex, and diabetes duration <4.9 years increasing probability of success to 74%. Six homogeneous subgroups were identified with different probabilities of achieving the composite outcome (Fig).

**Conclusion:** In summary, recursive partitioning identified individual characteristics and subgroups of patients predicting the response to therapy. Such analyses may guide clinicians in individualising treatment approaches.



Supported by: Novo Nordisk

## PS 063 GLP-1 based therapies

807

### Once-daily lixisenatide added on to consistently titrated insulin glargine plus oral agents in type 2 diabetes: the GetGoal-Duo 1 study

J. Rosenstock<sup>1</sup>, T. Forst<sup>2</sup>, R. Aronson<sup>3</sup>, L. Sauque Reyna<sup>4</sup>, E. Souhami<sup>5</sup>, S. Ping<sup>6</sup>, M. Riddle<sup>7</sup>;

<sup>1</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, USA,

<sup>2</sup>Institute for Clinical Research and Development, Mainz, Germany,

<sup>3</sup>LMC Endocrinology Centres, Toronto, Canada, <sup>4</sup>Unidad Metabólica y Cardiovascular SC de Cuernavaca, Mexico, <sup>5</sup>Sanofi, Paris, France, <sup>6</sup>Sanofi, Bridgewater, USA, <sup>7</sup>Oregon Health and Science University, Portland, USA.

**Background and aims:** Lixisenatide is a once-daily glucagon-like peptide 1 (GLP-1) receptor agonist with robust postprandial effects that could complement basal insulin therapy. This randomized, double-blind, multicentre trial in Type 2 diabetes mellitus (T2DM) inadequately controlled on metformin ± sulfonylureas ± thiazolidinediones (TZDs) assessed the efficacy and safety of lixisenatide added on to titrated insulin glargine + metformin ± TZDs.

**Materials and methods:** Glargine was initiated and titrated in a 12-week run-in phase to achieve fasting plasma glucose 4.4–5.6 mmol/L. People with HbA<sub>1c</sub> ≥7.0% and ≤9.0%, and whose mean fasting self-monitored plasma glucose was ≤7.8 mmol/L were then randomized to morning injections of once-daily lixisenatide 20 µg (n=223) or placebo (n=223) for 24 weeks while glargine titration continued. At randomization, sulfonylureas were stopped; all participants received metformin and 12% also received a TZD.

**Results:** Patient demographics were similar in both groups (mean age 56.2 years, T2DM duration 9.2 years, BMI 31.8 kg/m<sup>2</sup>). Primary endpoint was change in HbA<sub>1c</sub> from randomization. HbA<sub>1c</sub> initially decreased during run-in from 8.6 to 7.6%, and lixisenatide further reduced HbA<sub>1c</sub> by 0.71 versus 0.40% with placebo with consistent glargine titration at Week 24 (least squares [LS] mean difference: -0.32%; p<0.0001). Significantly more lixisenatide patients achieved HbA<sub>1c</sub> <7.0% (56 vs 39% with placebo; p=0.0001). Lixisenatide significantly improved 2-h postprandial glucose (PPG) after a standardized breakfast, and had a beneficial effect on body weight versus placebo despite titration of glargine doses (LS mean difference: -0.89 kg; p=0.0012). Adverse events (AEs) occurred in 80% of lixisenatide participants versus 68% on placebo; nausea/vomiting were more common with lixisenatide (27.4/9.4% vs 4.9/1.3%), as was discontinuation due to AEs (8.5 vs 3.6%). Nausea and vomiting were the most common AEs leading to discontinuation in the lixisenatide group (total of 4.0 vs 0.0% in the placebo group). Documented hypoglycaemia (blood glucose <3.3 mmol/L) was reported in 20.2% of the lixisenatide-treated patients versus 11.7% of those receiving placebo, with only 0.80 versus 0.44 events/patient/year, respectively. There was only one episode of severe hypoglycaemia in the lixisenatide group (0.4%) and none with placebo.

**Conclusion:** Lixisenatide added to consistently titrated insulin glargine plus oral agents significantly improved HbA<sub>1c</sub>, reduced PPG and had a beneficial effect on weight, with an expected GLP-1 AE profile that subsided over time.

Efficacy parameters in mITT population		Lixisenatide (n=223)	Placebo (n=223)
HbA <sub>1c</sub> (%)	Mean screening ± SD	8.60 ± 0.80	8.60 ± 0.80
	Mean baseline* ± SD	7.56 ± 0.54	7.60 ± 0.54
	Mean Week 24 ± SD	6.96 ± 0.81	7.30 ± 0.85
	LS mean ± SE change from baseline	-0.71 ± 0.09	-0.40 ± 0.09
	LS mean difference vs placebo (95% CI)	-0.32 (-0.463 to -0.171)	-0.40 ± 0.09
Proportion achieving HbA <sub>1c</sub> <7.0%	n (%)	121 (56.3%)	85 (38.5%)
	p value vs placebo	p=0.0001	
Proportion achieving HbA <sub>1c</sub> ≤6.5%	n (%)	69 (32.1%)	36 (16.3%)
	p value vs placebo	p<0.0001	
2-h PPG (mmol/L) <sup>†</sup>	Mean baseline* ± SD	13.02 ± 3.83	12.85 ± 3.75
	Mean Week 24 ± SD	9.87 ± 4.24	12.85 ± 3.75
	LS mean ± SE change from baseline	-3.09 ± 0.48	13.04 ± 3.94
	LS mean difference vs placebo (95% CI)	-3.16 (-3.951 to -2.375)	0.08 ± 0.48
Average 7-point SMPG (mmol/L)	Mean baseline* ± SD	8.20 ± 1.45	8.29 ± 1.52
	Mean Week 24 ± SD	7.75 ± 1.51	8.21 ± 1.72
	LS mean ± SE change from baseline	-0.47 ± 0.18	8.21 ± 1.72
	LS mean difference vs placebo (95% CI)	-0.39 (-0.680 to -0.107)	-0.08 ± 0.18
FPG (mmol/L)	Mean baseline* ± SD	6.56 ± 1.74	6.69 ± 1.98
	Mean Week 24 ± SD	6.70 ± 1.79	6.86 ± 1.88
	LS mean ± SE change from baseline	0.34 ± 0.21	0.46 ± 0.21
	LS mean difference vs placebo (95% CI)	-0.12 (-0.463 to 0.232); p=0.5142	

\*After 12 weeks of optimal titration of insulin glargine; <sup>†</sup>Using a standardized breakfast meal test; mITT=modified intention to treat; SD=standard deviation; LS=least squares; SE=standard error; CI=confidence interval; PPG=postprandial glucose; SMPG=self-monitored plasma glucose; FPG=fasting plasma glucose

Clinical Trial Registration Number: NCT00975286

Supported by: Sanofi

808

### Effects of lixisenatide once daily on gastric emptying and its relationship to postprandial glycaemia in type 2 diabetes mellitus

M. Lorenz, C. Pfeiffer, A. Steinsträßer, P. Ruus, R. Becker;

Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany.

**Background and aims:** Lixisenatide is a glucagon-like peptide-1 (GLP-1) receptor agonist intended for the treatment of patients with Type 2 diabetes mellitus (T2DM). Lixisenatide blunts postprandial blood glucose (PPBG) excursions by delaying gastric emptying in addition to stimulating glucose-dependent insulin release and suppressing glucagon secretion. This study evaluated the relationship between the gastric emptying rate and PPBG-area under the curve (AUC) values after intake of mixed meals in patients with T2DM receiving lixisenatide 20 µg once daily (QD) or placebo in the morning.

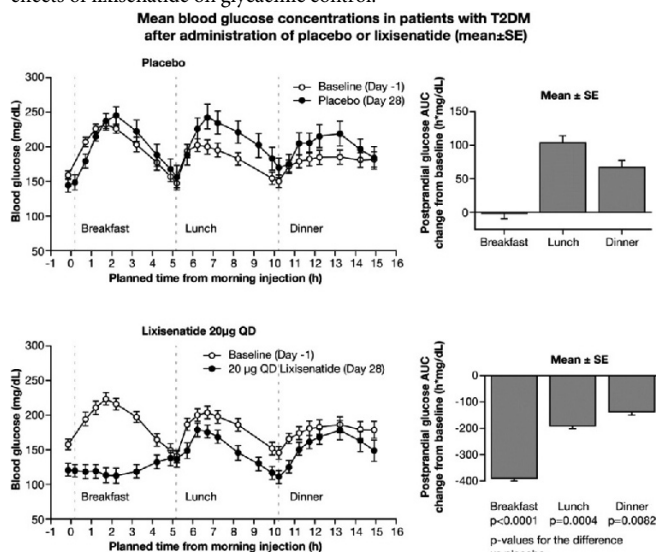
**Materials and methods:** Data were obtained from a 28-day, randomized, double-blind, placebo-controlled, parallel-group study (lixisenatide n=19; placebo n=22) in patients with T2DM on top of up to two oral antidiabetic agents. Lixisenatide was injected subcutaneously QD using an ascending dose range beginning at 5 µg and increasing in increments of 2.5 µg over 4-day intervals to a final dose of 20 µg QD from Day 25 to Day 28. In addition to 24-hour blood glucose monitoring, a <sup>13</sup>C-octanoic acid breath test was conducted to explore the gastric emptying rate during a standardized breakfast at Day 28.

**Results:** For lixisenatide 20 µg QD in the morning, the change in PPBG-AUC on Day 28 corrected for values at baseline (Day -1 prior to initiation of study treatment) was significantly reduced compared with placebo after a standardized breakfast (p<0.0001; Figure), after lunch (p=0.0004) and after dinner (p=0.0082). Thus, lixisenatide 20 µg QD administered in the morning demonstrated a pharmacodynamic effect on blood glucose levels after all meals throughout the day. The mean gastric emptying half-life (t<sub>1/2</sub>) was significantly longer for lixisenatide 20 µg compared with placebo (p=0.0031); mean (± standard deviation) changes from baseline for t<sub>1/2</sub> were -24 ± 133 minutes for placebo (n=17) and 212 ± 279 minutes for lixisenatide (n=17). There was an inverse relationship between PPBG and gastric emptying rate for lixisenatide after the standardized breakfast; linear regression of PPBG-AUC<sub>0.14–4.55h</sub> versus t<sub>1/2</sub> (n=17, r<sup>2</sup>=0.4889; p=0.0018).

**Conclusion:** Lixisenatide 20 µg QD injected subcutaneously in the morning significantly slows gastric emptying accompanied by a significant PPBG re-



duction, which is considered to be an important mechanism for the beneficial effects of lixisenatide on glycaemic control.



T2DM=Type 2 diabetes mellitus; SE=standard error; QD=once daily; AUC=area under the curve

Supported by: Sanofi

## 809

### Anti-atherosclerotic activity of lixisenatide in ApoE knockout mice

T. Hübschle, H.-L. Schäfer, H.-P. Juretschke, H. Rütten, U. Werner; Sanofi-Aventis Deutschland GmbH, Translational Medicine, Frankfurt am Main, Germany.

**Background and aims:** The ApoE knockout (KO) mouse develops atherosclerotic plaques with morphology resembling human atherosclerosis. This model was used to investigate the effects of chronic lixisenatide treatment on atherosclerotic plaque formation.

**Materials and methods:** Male ApoE KO mice (B.129P2-apoe<sup>tm1Unc/J</sup>) were treated for 16 weeks by continuous infusion via subcutaneous osmotic minipumps (ALZET™). Animals received 3.6 µg/day for the first 4 weeks and 5.04 µg/day for the subsequent 12 weeks. ApoE KO control mice received the same volume of placebo. Untreated mice from the background strain (C57BL/6J) were used as a second, healthy control.

**Results:** Total serum cholesterol and blood glucose were significantly reduced by lixisenatide both during (-41 and -10%, respectively, Day 35) and at the end of treatment (-42 and -13%, respectively, Day 112). The decrease in total serum cholesterol was related to a decrease in atherogenic lipoprotein fractions. Treatment with lixisenatide had no significant effect on relative liver weight, hepatic cholesterol, triglyceride or phospholipid concentrations at study end. After 16 weeks of lixisenatide treatment, three methodologically independent measures (invasive [macroscopic and histology] and non-invasive [ultra-small superparamagnetic iron oxide (USPIO)-based magnetic resonance imaging (MRI)]) were used to quantify atherosclerotic plaque formation. When compared with the wild-type background, ApoE KO mice receiving placebo developed atherosclerotic lesions at the total inner surface of the aorta and the aortic root semilunar valve region of the heart. In contrast, lixisenatide treatment in ApoE KO mice resulted in a significant reduction of atherosclerotic plaque formation of ~30% relative to placebo. In more detail, lixisenatide significantly reduced atherosclerotic lesions at the total inner surface of the aorta by 27% (oil red staining) and at the aortic root semilunar valve region by 29% (Movat-Pentachrome staining) or 30% (USPIO-based MRI).

**Conclusion:** Lixisenatide was associated with robust anti-atherosclerotic activity with a significant reduction of atherogenic lipoproteins in ApoE KO mice.

Supported by: Sanofi

## 810

### Efficacy and safety of lixisenatide once daily versus placebo in patients with type 2 diabetes insufficiently controlled on pioglitazone (GetGoal-P)

M. Pinget<sup>1</sup>, R. Goldenberg<sup>2</sup>, E. Niemoeller<sup>3</sup>, I. Muehlenbartmer<sup>4</sup>, R. Aronson<sup>5</sup>;

<sup>1</sup>University Hospital Strasbourg, France, <sup>2</sup>LMC Diabetes and Endocrinology, Thornhill, Canada, <sup>3</sup>Sanofi-aventis Deutschland GmbH, Frankfurt am Main, Germany, <sup>4</sup>Sanofi, Paris, France, <sup>5</sup>LMC Endocrinology Centres, Toronto, Canada.

**Background and aims:** Lixisenatide is a once-daily glucagon-like peptide-1 (GLP-1) receptor agonist for the treatment of Type 2 diabetes mellitus (T2DM). The aim of the GetGoal-P study was to evaluate the efficacy and safety of lixisenatide (LIXI) as add-on therapy in patients with T2DM insufficiently controlled by ≥30 mg/day pioglitazone ± metformin.

**Materials and methods:** GetGoal-P was a randomized, double-blind, placebo-controlled, multicentre study comparing the efficacy and safety of lixisenatide 20 µg once daily versus placebo (PBO). The primary objective was the absolute HbA<sub>1c</sub> reduction with LIXI versus PBO at Week 24 (main treatment period).

**Results:** At baseline, mean age was 55.8 years old, mean diabetes duration 8.1 years, mean HbA<sub>1c</sub> 8.1% and mean BMI 33.9 kg/m<sup>2</sup>. LIXI once daily produced a significantly greater HbA<sub>1c</sub> reduction versus placebo (Table) and a greater proportion of patients achieved HbA<sub>1c</sub> <7.0% (52% LIXI vs 26% PBO; p<0.0001). Significantly fewer patients receiving LIXI required rescue therapy (3.8 vs 11.3% PBO). Overall, LIXI was well tolerated, with a similar proportion of adverse events and serious adverse events (72.4 and 2.5% in the LIXI group versus 72.7 and 1.9% in the placebo group). Only 6.5% of LIXI and 5.0% of PBO-treated patients discontinued due to adverse events at Week 24, mainly related to gastrointestinal events (Table). Symptomatic hypoglycaemia rates were low in both groups (Table). There were no cases of severe hypoglycaemia in either group.

**Conclusion:** In conclusion, in patients with T2DM insufficiently controlled on pioglitazone ± metformin, LIXI 20 µg once daily significantly improved glycaemic control with a low risk of hypoglycaemia and was well tolerated over 24 weeks.

Efficacy parameters in mITT population		Lixisenatide 20 µg once daily (n=320)	Placebo (n=159)
HbA <sub>1c</sub> (%)	Mean baseline ± SD	8.08 ± 0.91	
	LS mean ± SE change from baseline	-0.90 ± 0.09	8.05 ± 0.78
	LS mean difference vs placebo	-0.56 (-0.73 to -0.39)	-0.34 ± 0.10
		p<0.0001	
Fasting plasma glucose (mmol/L)	Mean baseline ± SD	9.14 ± 2.15	
	LS mean ± SE change from baseline	-1.16 ± 0.19	9.12 ± 2.19
	LS mean difference vs placebo	-0.84 (-1.21 to -0.47)	-0.32 ± 0.22
		p<0.0001	
Body weight (kg)	Mean baseline ± SD	92.8 ± 23.0	
	LS mean ± SE change from baseline	-0.21 ± 0.32	97.0 ± 25.8
	LS mean difference vs placebo	-0.41 (-1.03 ± 0.20)	0.21 ± 0.36
		p=0.1864	
Safety parameters in safety population, N (%)			
Symptomatic hypoglycaemia <sup>a</sup>		11 (3.4%)	2 (1.2%)
Nausea		76 (23.5%)	17 (10.6%)
Vomiting		22 (6.8%)	6 (3.7%)
Diarrhoea		23 (7.1%)	17 (10.6%)
Event with clinical symptoms with either plasma glucose <3.3 mmol/L or prompt recovery after oral carbohydrate administration if no plasma glucose measurement was available; mITT=modified intention to treat; SD=standard deviation; LS=least squares; SE=standard error			

\*Event with clinical symptoms with either plasma glucose <3.3 mmol/L or prompt recovery after oral carbohydrate administration if no plasma glucose measurement was available; mITT=modified intention to treat; SD=standard deviation; LS=least squares; SE=standard error

Clinical Trial Registration Number: NCT00763815

Supported by: Sanofi

## 811

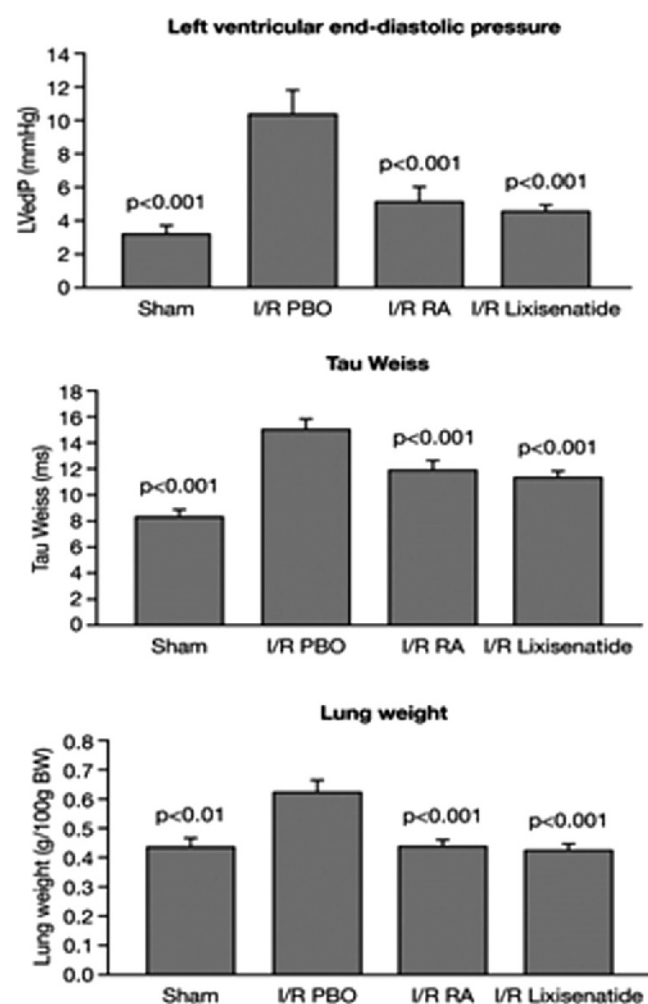
**Cardioprotective effect of chronic treatment with lixisenatide in an *in vivo* rat model of myocardial ischaemia/reperfusion-induced injury**P. Wohlfart<sup>1</sup>, W. Linz<sup>1</sup>, D. Linz<sup>2</sup>, T. Hübschle<sup>1</sup>, U. Werner<sup>1</sup>, H. Ruetten<sup>1</sup>;<sup>1</sup>Sanofi-Aventis Deutschland GmbH, Translational Medicine, Frankfurt am Main, <sup>2</sup>Saarland University – Faculty of Medicine, Homburg/Saar, Germany.

**Background and aims:** Effects of glucagon-like peptide-1 receptor (GLP-1R) agonists on the cardiovascular system have been described recently. In isolated rat hearts, the GLP-1R agonist lixisenatide significantly reduced infarct size after ischaemia reperfusion (I/R) *ex vivo*. In this study, the effect of chronic lixisenatide treatment versus placebo on cardiovascular outcome after ischaemia (30 minutes) and long-term reperfusion of the left coronary artery was investigated *in vivo*.

**Materials and methods:** Male Wistar rats (250–300 g) were randomized into four groups: 1) sham-operated controls without I/R (Sham); 2) I/R placebo; 3) I/R ramipril (1 mg/kg/day in chow); 4) I/R lixisenatide (10 µg/kg/day subcutaneously via osmotic mini-pump). After 10 weeks of treatment, heart function was investigated via tip catheter, an autopsy performed and the lungs were weighed. Plasma brain natriuretic peptide (BNP) was determined as a marker of chronic heart failure.

**Results:** Lixisenatide significantly attenuated I/R-induced deterioration of diastolic function (left ventricular end-diastolic pressure) and Tau Weiss, a measure of myocardial relaxation (Figure). Lung weight was significantly increased with placebo (a measure of congestion), but was normalized by lixisenatide and ramipril (Figure). Moreover, BNP levels were significantly ( $p<0.05$ ) reduced by either agent. Thus, deterioration of left ventricular function following I/R was significantly prevented by chronic lixisenatide treatment.

**Conclusion:** In conclusion, lixisenatide has shown, in a rat model of myocardial I/R-induced injury, cardioprotective effects comparable with the angiotensin-converting enzyme inhibitor ramipril.



Supported by: Sanofi

## 812

**Absence of calcitonin response in GLP-1R knock-out mice after treatment with lixisenatide**T. Kissner<sup>1</sup>, L. Essermeant<sup>2</sup>, J.-F. Gallas<sup>2</sup>, G. Hadour<sup>2</sup>;<sup>1</sup>Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany, <sup>2</sup>Sanofi, Montpellier, France.

**Background and aims:** Proliferative C-cell findings in rodent thyroid have been reported in long-term pre-clinical studies with glucagon-like peptide-1 receptor (GLP-1R) agonists. The association between calcitonin level and thyroid C-cell proliferation has been recently debated. Plasma calcitonin is increased in association with C-cell proliferation in rodents at very high doses and also in shorter-term studies without C-cell proliferation. Using GLP-1R knockout (KO) mice, we investigated the hypothesis that calcitonin release at high lixisenatide doses is mediated via GLP-1R activation.

**Materials and methods:** Male and female CD-1 (wild-type) mice and GLP-1R (-/-) KO mice based on CD-1 genetic background were given high subcutaneous (SC) doses of lixisenatide 1000 µg/kg twice daily or control vehicle for 14 days (n=8/group). Plasma calcitonin concentration was measured by immune-radiometric assay 2 hours after last administration on Day 14. As 50% of calcitonin values were under the lower limit of quantification (10 pg/mL), an exact pairwise Wilcoxon's test was used to analyse between-group differences.

**Results:** In CD-1 mice, lixisenatide treatment resulted in statistically significant higher mean plasma calcitonin concentrations versus control (Table;  $p<0.001$  for pooled male/female animals). No lixisenatide-induced calcitonin release was noted in GLP-1R (-/-) KO mice ( $p$ =non-significant). In this group, the plasma calcitonin concentration remained low and similar to values observed in CD-1 and control GLP-1R (-/-) KO mice. Moreover, at this dose level, no C-cell proliferation was seen in studies up to 3 months in CD-1 mice.

**Conclusion:** The absence of lixisenatide-induced calcitonin release in GLP-1R (-/-) KO mice, in contrast to wild-type mice, suggests that the GLP-1R is involved in the rodent thyroid pathway of C-cell activation and causes calcitonin release. In addition, in this study calcitonin increase was not associated with thyroid C-cell proliferation.

Mean calcitonin levels in CD-1 (wild-type) and GLP-1R (-/-) KO mouse plasma on Day 14

Animal strain and treatment	Gender	Calcitonin concentration (pg/mL) on Day 14 (Mean ± SD)
CD-1 (wild-type), Control vehicle	Female (n=6)	<16.6 ± NA
	Male (n=2)	<15 ± NA
CD-1 (wild-type), Lixisenatide 1000 µg/kg twice daily	Female (n=5)	99.8 ± 50.9
	Male (n=3)	86.6 ± 29.8
GLP-1R (-/-) KO, Control vehicle	Female (n=6)	10.7 ± NA
	Male (n=2)	<18.8 ± NA
GLP-1R (-/-) KO, Lixisenatide 1000 µg/kg twice daily	Female (n=5)	<10.1 ± NA
	Male (n=3)	<16.5 ± 8.7

For values below the lower limit of quantification (LLOQ), LLOQ was used to calculate the maximum bound for the mean; NA=not applicable, as either all values or more than 50% of values were below the LLOQ; GLP-1R=glucagon-like peptide-1 receptor; KO=knockout; SD=standard deviation

Supported by: Sanofi

## 813

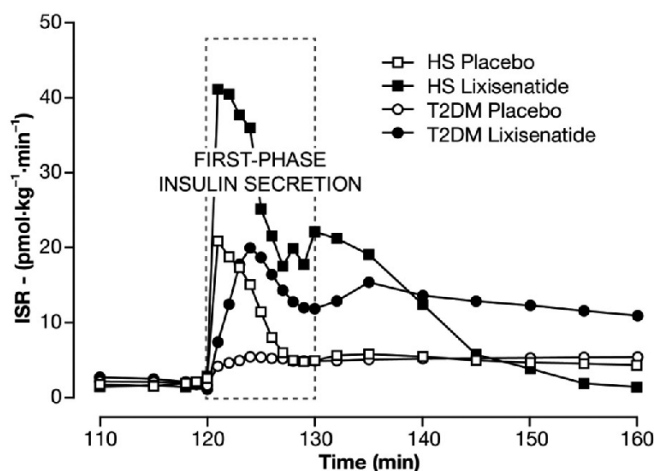
**Restitution of glucose disposition with lixisenatide in subjects with type 2 diabetes**R.H. Becker<sup>1</sup>, C. Kapitza<sup>2</sup>, J. Stechl<sup>1</sup>, P. Ruus<sup>1</sup>, J. Msihid<sup>3</sup>;<sup>1</sup>Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany, <sup>2</sup>Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany, <sup>3</sup>Sanofi, Paris, France.

**Background and aims:** Restitution of glucose disposition is a therapeutic goal in Type 2 diabetes mellitus (T2DM). We compared insulin secretion (IS) and glucose disposition with lixisenatide (LIXI) in early-stage T2DM and non-diabetic healthy subjects.

**Materials and methods:** In two single-centre, two-period, two-treatment, two-sequence, single-dose, crossover studies, healthy subjects (14 male/six female, mean age 36 years old, BMI 25 kg/m<sup>2</sup>) and patients with T2DM (13 male/nine female, mean age 55 years old, BMI 30 kg/m<sup>2</sup>) received LIXI 20 µg subcutaneously or placebo (PBO) 2 hours prior to an intravenous glucose challenge (IVG; 0.3 g/kg over 30 seconds). Integration of IS rate (ISR) by C-peptide deconvolution was used to determine first- (ISR-AUC<sub>0-10min</sub>) and second- (ISR-AUC<sub>10-120min</sub>) phase insulin secretion. Insulin, C-peptide and glucagon concentration-time curves and glucose disposition rate ( $K_{glu}$ ) were determined for 2 hours.

**Results:** LIXI enhanced first-phase insulin secretion in T2DM to PBO levels in healthy subjects, while second-phase insulin secretion was greater and elevated for >2 hours in subjects with T2DM, in both PBO and LIXI groups (Figure). Insulin and C-peptide were correspondingly greater.  $K_{glu}$  was accelerated twofold by LIXI, but did not fully recover to values in healthy subjects. When insulin levels changed, glucagon levels followed inversely and glucose was brought to normal levels.

**Conclusion:** A single dose of LIXI 20 µg quantitatively restored first-phase insulin release to IVG in subjects with T2DM, enlarged second-phase insulin secretion and enhanced glucose disposition towards normal values, without impairing counter-regulation by glucagon.



ISR=insulin secretion rate; HS=healthy subjects; T2DM=Type 2 diabetes mellitus

Supported by: Sanofi

## 814

### Augmentation of first-phase insulin release with lixisenatide in non-diabetic subjects

J. Stechl<sup>1</sup>, R.H. Becker<sup>1</sup>, C. Kapitza<sup>2</sup>, J. Msihid<sup>3</sup>;

<sup>1</sup>Sanofi, Frankfurt am Main, Germany, <sup>2</sup>Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany, <sup>3</sup>Sanofi, Paris, France.

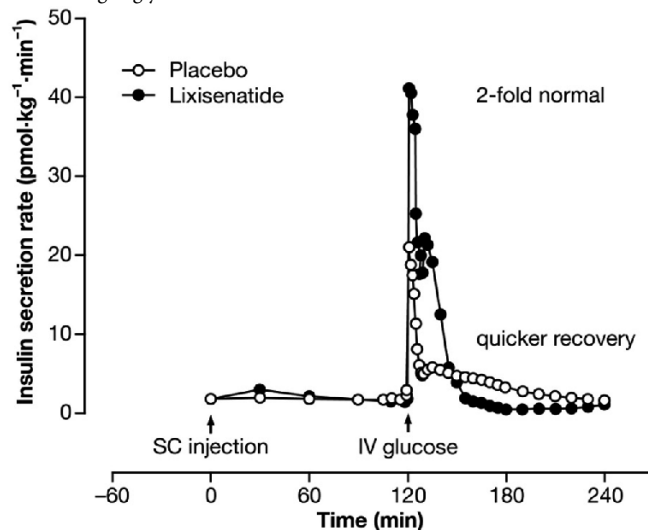
**Background and aims:** Lixisenatide restores insulin release and accelerates glucose disposition in Type 2 diabetes. We investigated the physiological response in non-diabetic subjects.

**Materials and methods:** In a two-period, two-treatment, two-sequence, single-centre, single-dose, crossover study, subjects (14 male, six female, mean age 36 years old, BMI 24 kg/m<sup>2</sup>) received single doses of lixisenatide 20 µg subcutaneously (SC) or matching placebo 2 hours prior to an intravenous glucose challenge (IVG; 0.3 g/kg over 30 seconds). First- (AUC<sub>0-10min</sub>) and second- (AUC<sub>10-120min</sub>) phase insulin secretion was integrated from the insulin secretion rate (based on C-peptide measurement).

**Results:** Maximum plasma lixisenatide concentration ( $C_{max}$  145 pg/mL; coefficient of variance [CV%] 44) occurred 2 hours (range 1–3 hours) after injection, with weak transient effects on fasting blood glucose and pre-IVG insulin levels. Lixisenatide enhanced first-phase insulin secretion 2.4-fold (90% confidence interval [CI]: 2.1–2.6), while second-phase release was unchanged overall, 0.9-fold (90% CI: 0.8–1.0), but was elevated up to 30 minutes and subsequently dropped sharply to baseline, differing significantly from placebo, which remained elevated. First- and second-phase insulin concentration increased 3.2-fold (90% CI: 2.7–3.8) and 3.4-fold (90% CI: 2.7–4.2), followed by 2.3-fold (90% CI: 1.9–3.0) accelerated glucose disposition, reducing blood glucose below counter-regulatory thresholds (<3.9 mmol/L) in some subjects.

Glucagon suppression was initially augmented and recovered faster in those with blood glucose <3.9 mmol/L.

**Conclusion:** A single dose of lixisenatide 20 µg SC boosts insulin release to IVG and accelerates glucose disposition in non-diabetic subjects without affecting the counter-regulatory interplay of insulin and glucagon release, thus maintaining euglycaemia.



Supported by: Sanofi

## 815

### Efficacy and safety of lixisenatide in elderly (≥65 years) and very elderly (≥75 years) patients with type 2 diabetes: an analysis from the GetGoal Phase 3 programme

D. Raccach<sup>1</sup>, P. Miossec<sup>2</sup>, V. Esposito<sup>2</sup>, E. Niemoeller<sup>3</sup>, M. Cho<sup>4</sup>, J. Gerich<sup>5</sup>;

<sup>1</sup>Hôpital Sainte Marguerite, Marseille, France, <sup>2</sup>Sanofi R&D, Paris, France, <sup>3</sup>Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany, <sup>4</sup>Sanofi, Bridgewater, USA, <sup>5</sup>Department of Medicine, University of Rochester School of Medicine, Rochester, USA.

**Background and aims:** The number of elderly people with Type 2 diabetes mellitus (T2DM) is increasing due to improved life expectancy, and managing elderly patients with T2DM can be challenging. The effect of age on the pharmacokinetics of lixisenatide was assessed in a study that included 18 elderly healthy subjects (age range 65–79 years) and 18 young healthy subjects (age range 24–44 years). The mean exposure after a 20 µg single dose was ~30% higher in elderly than in young subjects and the terminal half-life was prolonged ~1.6 times.  $C_{max}$  and  $t_{max}$  were comparable in both groups. The aim of this analysis was to compare clinical outcomes for lixisenatide across age groups in the Phase III clinical trial programme.

**Materials and methods:** To date, 612 elderly patients (aged ≥65 years) overall have been exposed to lixisenatide in Phase II/III clinical trials (567.9 patient-years), including 59 very elderly (aged ≥75 years) patients (58.3 patient-years). This analysis of six randomized, placebo-controlled Phase III trials assessed the efficacy (HbA<sub>1c</sub>) and safety (overall adverse events, gastrointestinal events, hypoglycaemia) of 20 µg once-daily lixisenatide in 379 patients aged ≥65 years, including 48 patients aged ≥75 years (436.5 and 49.1 patient-years' exposure, respectively) during the main 12-week (GetGoal-Mono) or 24-week (GetGoal-M, -F1, -S, L, -L-Asia) treatment periods.

**Results:** The efficacy profiles were similar regardless of age, with comparable HbA<sub>1c</sub> decreases in patients aged ≥65 years versus patients aged <65 years for lixisenatide in all six studies, and significantly greater decreases versus placebo in both age categories. In a pooled analysis of the six studies, the lixisenatide safety profile was also comparable regardless of age, with overall adverse events and gastrointestinal event incidences in lixisenatide-treated patients of 72 and 43% in patients aged ≥65 years versus 69 and 41% in patients aged <65 years, and 73 and 46% in patients aged ≥75 years versus 69 and 41% in patients aged <75 years. The incidence of symptomatic hypoglycaemic events varied depending on the background treatment and was usually comparable between lixisenatide and placebo and no relevant difference between age categories was observed.

**Conclusion:** In conclusion, in this analysis, lixisenatide was shown to be effective and well tolerated in elderly and very elderly patients.

Supported by: Sanofi



## 816

**C-peptide response to glucagon challenge predicts improvement by liraglutide of both early insulin secretion and glucose tolerance**T. Matsuda<sup>1</sup>, M. Takabe<sup>1</sup>, Y. Hirota<sup>1</sup>, N. Hashimoto<sup>1</sup>, T. Nakamura<sup>1</sup>, K. Sakaguchi<sup>1</sup>, W. Ogawa<sup>1</sup>, S. Seino<sup>1,2</sup>;<sup>1</sup>Division of Diabetes and Endocrinology, <sup>2</sup>Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan.

**Background and aims:** Liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, provides effective treatment for glycaemic control in type 2 diabetes mellitus (T2DM). The beneficial effects of liraglutide vary by individual, but little knowledge of clinical parameters to predict its effect is available. In this study, we investigated predictive factors for the effects of liraglutide on insulin secretion and glucose excursion in patients with T2DM.

**Materials and methods:** Patients with T2DM (N=24) were hospitalised and treated for glucose toxicity with oral hypoglycaemic agents and/or insulin. After achieving the target level of glycaemic control (fasting plasma glucose <130 mg/ dL, postprandial plasma glucose <180 mg/ dL), liraglutide treatment was started at a dose of 0.3 mg/day, and was gradually increased to 0.9 mg/day (the maximum dose approved in Japan) if tolerated. The final dose and treatment duration of liraglutide was  $0.79 \pm 0.19$  mg/day and  $17.5 \pm 1.7$  days (mean  $\pm$  SD), respectively. A 75g-oral glucose tolerance test (OGTT) and a glucagon challenge test (in which 1mg of glucagon was administered intravenously and the serum levels of CPR before and 6 min after the challenge were measured) were performed before treatment; a second OGTT was performed after treatment to assess the effects of liraglutide. The long-term effect of the drug also was assessed by the change in HbA1c level.

**Results:** Liraglutide improved glucose excursion without significant increase in total insulin secretion [area-under-the curve of plasma insulin (AUC insulin)] during OGTT [before treatment (before),  $4920 \pm 4735$   $\mu$ U/ml; after treatment (after),  $5958 \pm 3866$   $\mu$ U/ml,  $P=0.16$ ]. On the other hand, Insulinogenic Index (I.I.) (before,  $0.16 \pm 0.36$ ; after,  $0.57 \pm 0.55$ ,  $P=0.001$ ) and serum insulin level at 30 min (before,  $27.9 \pm 27.9$   $\mu$ U/ml; after,  $37.6 \pm 35.3$   $\mu$ U/ml,  $P=0.01$ ) and 60 min (before,  $40.5 \pm 38.6$   $\mu$ U/ml; after,  $63.9 \pm 65.1$   $\mu$ U/ml,  $P=0.02$ ) during OGTT were significantly increased by liraglutide. Serum CPR levels 6 min after glucagon administration (CPR 6min) showed the greatest correlation coefficient ( $r=0.92$ ) among the various parameters of insulin secretion obtained before treatment, including HOMA-beta index, fasting serum CPR level, ratio of fasting serum CPR to plasma glucose level, insulinogenic index, AUC insulin during OGTT, difference of serum CPR level before and after glucagon challenge test, and serum CPR after 6 min after glucagon challenge test (CPR 6min). When the effect of liraglutide in patients was categorised into tertiles by CPR 6min, the highest responders to the glucagon challenge test showed the greatest improvement in terms of early insulin response and glucose excursion during OGTT, and also in reduction of HbA1c level.

**Conclusion:** Improvement of glucose tolerance by liraglutide in T2DM patients is associated with increased early insulin secretion but not total insulin secretion (AUC insulin). Glucagon-stimulated CPR secretion may be a useful predictor for the effects of liraglutide in T2DM.

**PS 064 Incretin related novel therapies**

## 817

**Discovery of a novel potent and selective TGR5 agonists with anti-diabetic effect**

M. Ning, H. Duan, S. Huang, J. Shen, Y. Leng;

Shanghai Institute of Material Medica, Chinese Academy of Sciences, Shanghai, China.

**Background and aims:** TGR5 is a member of G-protein coupled receptor which coupled to the formation of cAMP that mediates various cellular and physiological effects. Activation of this receptor will promote the production of glucagons-like peptide (GLP-1) and upregulate genes which involved in the control of energy expenditure. Those advantages made TGR5 as an attractive target for the treatment of diabetes, obesity, and metabolic syndrome. In the present study, a novel potent and selective TGR5 agonist was discovered and its *in vivo* anti-diabetic effect was evaluated.

**Materials and methods:** The *in vitro* activation of human and mouse TGR5 was determined by cell based reporter gene assay in hTGR5/CRE/HEK293 and mTGR5/CRE/HEK293 cell lines. Glucose tolerance test and GLP-1 secretion was performed on ICR mice. The acute and chronic anti-diabetic effect was investigated in db/db and ob/ob mice.

**Results:** MN6 was discovered as a potent and selective TGR5 agonist with  $EC_{50}$  of 1.55 nM on human TGR5 and 17.8 nM on mouse TGR5, but no activation of FXR. Single oral dose of 50 mg/kg MN6 significantly decreased blood glucose levels following an OGTT in ICR mice, and caused 44.8% reduction of the area under the curve ( $AUC_{0-120min}$  Glu). Moreover, an acute administration of MN6 also enhanced both basal or glucose induced GLP-1 release in ICR mice. Single oral dose of 25 or 50 mg/kg MN6 decreased blood glucose levels in db/db mice, and the chronic administration of MN6 could significantly decrease the HbA1c levels in ob/ob mice.

**Conclusion:** MN6 is a novel and selective TGR5 receptor agonist, which showed good potency in both *in vitro* and *in vivo* assays. It might be a therapeutic candidate for the treatment of T2DM and metabolic syndrome.

## 818

**Glucagon receptor antagonist LY2409021 does not delay recovery from insulin induced hypoglycaemia in patients with type 2 diabetes mellitus**R. Kelly<sup>1</sup>, C.N. Lim<sup>1</sup>, E. Pratt<sup>1</sup>, M.T. Loh<sup>1</sup>, M. Deeg<sup>2</sup>, H. Fu<sup>2</sup>, S. Cui<sup>3</sup>, P. Garhyan<sup>2</sup>;<sup>1</sup>Eli Lilly, Singapore, <sup>2</sup>Eli Lilly, Indianapolis, USA, <sup>3</sup>Pharmanet/i3, Ann Arbor, USA.

**Background and aims:** In patients (pts) with type 2 diabetes mellitus (T2DM), glucagon receptor antagonism improves hyperglycaemia by inhibiting hepatic glucose output. It has not been studied whether an appropriate physiologic response to hypoglycaemia is preserved in the presence of a glucagon receptor blockade. LY2409021 (LY) is a selective and potent glucagon receptor antagonist. Our primary aim was to determine if recovery from hypoglycaemia in pts with T2DM is impeded by administration of LY vs. placebo (PB). Our secondary aim was to examine responses of counter-regulatory hormones under these conditions.

**Materials and methods:** Thirteen Asian pts (21 to 65 years) with T2DM ( $HbA_{1c} \leq 11.0\%$ ), half of whom were taking metformin (Met) (n=7), were enrolled in this single-site, single-dose, subject-blind study. Pts were randomised to 1 of 2 treatment sequences and received PB or 1 dose of 90 mg LY (a dose expected to achieve near maximal glucagon receptor antagonism) 12 hours before insulin infusion to achieve hypoglycaemia (blood glucose  $\approx 40$  mg/dL).

**Results:** With LY vs. PB, the LS mean insulin load required to achieve hypoglycemia was 1920 pmol/kg vs. 2170 pmol/kg. Mean time to recovery from hypoglycaemia to a blood glucose  $\geq 63$  mg/dL was similar between LY and PB, and among pts taking Met and not taking Met (Table). During hypoglycaemia, mean glucagon levels were 2- to 3-fold higher after LY than after PB. The AUC and  $C_{max}$  of epinephrine, norepinephrine, cortisol, and growth hormone responses to hypoglycaemia were comparable between groups. No deaths or serious adverse events occurred during the study.

**Conclusion:** LY does not hamper recovery from insulin-induced hypoglycaemia in pts with T2DM.

## Mean time to recovery from hypoglycaemia

	Overall		Met*		No Met	
	LY N=12	PB N=12	LY N=6	PB N=6	LY N=6	PB N=6
<b>LS mean time to recovery</b>	58.19	53.45	51.40	52.65	65.87	54.26
<b>Mean Ratio</b>	1.09		0.98		1.21	
<b>90% CI</b>	0.88, 1.35		0.71, 1.33		0.90, 1.64	
<b>P-value</b>	0.4928		0.8916		0.2720	

**Abbreviations:** LS=least squares, LY=LY2409021, Met=metformin, PB=placebo.

\*One patient taking Met was not included in the analysis due to lack of post-baseline data.

Supported by: Eli Lilly and Company

## 819

## Functional characterisation of a mouse-selective small molecule agonist of TGR5

M.D. Michael<sup>1</sup>, X. Ruan<sup>1</sup>, C.C. Cheng<sup>1</sup>, A.M. Siesky<sup>1</sup>, C. Dominguez<sup>2</sup>, S.G. Sanfeliciano<sup>2</sup>, C. Montero<sup>2</sup>, C.S. Suen<sup>1</sup>, Y. Xu<sup>1</sup>, D.A. Briere<sup>2</sup>;

<sup>1</sup>Lilly Research Laboratories, Indianapolis, <sup>2</sup>Lilly Research Laboratories, Alcobendas, Spain.

**Background and aims:** TGR5 (also called GPR109A and GPR131) is a G-protein coupled receptor that is expressed in intestine, brown adipose tissue (BAT), and gallbladder and is activated by bile acids. Activation of TGR5 in intestine stimulates cyclic AMP (cAMP) resulting in release of glucagon-like peptide-1 (GLP-1) from enteroendocrine cells, whereas activation of TGR5 in BAT has been reported to increase energy expenditure. In addition, activation of TGR5 has been recently shown to increase gallbladder filling with bile. Currently, the only published agonists of TGR5 are bile acids or bile acid analogs, which have demonstrated small increases on acute GLP-1 secretion, little to no effects on acute glucose tolerance, and small increases in gallbladder volume *in vivo*. Here we present the effects of a novel, non-steroidal small molecule agonist of TGR5 on GLP-1 and peptide YY (PYY) secretion, glucose tolerance, and gallbladder filling.

**Materials and methods:** cAMP and GLP-1 production were measured in compound 8440 stimulated STC-1 and NCI-H716 cells using Cisbio HTRF cAMP and Mesoscale GLP-1 kits. Wild-type C57BL/6 mice or TGR5 knock-out mice were orally dosed for 1–3 days with compounds with and without glucose, followed by timed measurements of glucose, insulin, and/or GLP-1. Gallbladder filling was measured by weighing bile from the gallbladder.

**Results:** In mouse enteroendocrine STC-1 cells, compound 8440 demonstrated potent cAMP production (580 nM EC<sub>50</sub>, 25-fold stimulation) and GLP-1 secretion (307 nM EC<sub>50</sub>, 5.7-fold stimulation). In human enteroendocrine NCI-H716 cells, compound 8440 demonstrated weak cAMP production (3095 nM EC<sub>50</sub>, 3.2-fold stimulation) and GLP-1 secretion (2656 nM EC<sub>50</sub>, 1.5-fold stimulation). Together, these data demonstrate that compound 8440 is a mouse-selective TGR5 agonist. In C57BL/6 mice, compound 8440 dose-dependently and time-dependently increased GLP-1 and PYY secretion. When dosed to C57BL/6 mice 15 min prior to an oral glucose tolerance test, compound 8440 caused a lowering of the glucose excursion, as well as superior and prolonged increases in GLP-1 and PYY, as compared to the dipeptidyl peptidase-4 inhibitor, sitagliptin. In addition, compound 8440 showed a dose-dependent increase in bile weight that could not be separated from doses that induced significant effects on GLP-1 secretion, PYY secretion, or lowering of glucose excursions in an oral glucose tolerance test. Lastly, compound 8440 failed to show any effects on GLP-1, PYY, and bile weight in TGR5 knockout mice.

**Conclusion:** These results demonstrate that compound 8440, a mouse-selective, non-steroidal TGR5 agonist, acutely induces significant GLP-1 and PYY secretion which leads to a lower glucose excursion in an oral glucose tolerance test. Importantly, the doses that reduce glucose excursion cannot be separated from doses that induce gallbladder filling. Overall, these data highlight the benefits and risks of using TGR5 agonists to treat diabetes and metabolic diseases.

## 820

## Effects of bile acid enemas on GLP-1 and PYY secretion in healthy humans

M.J. Bound<sup>1</sup>, T. Wu<sup>1</sup>, B. Gedulin<sup>2</sup>, M. Horowitz<sup>1</sup>, C.K. Rayner<sup>1</sup>;

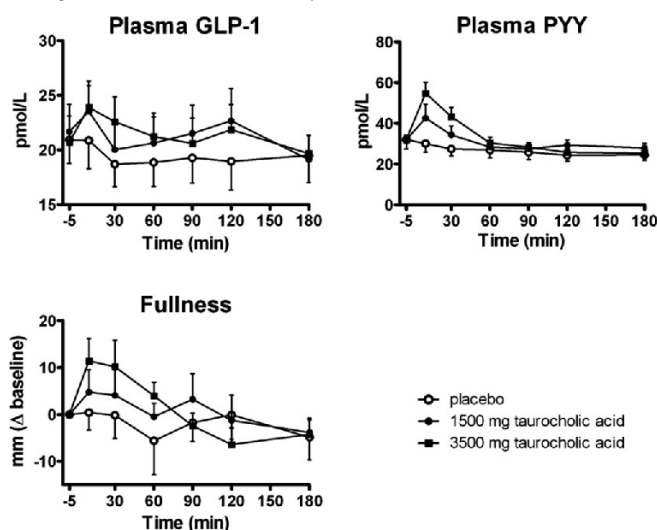
<sup>1</sup>Discipline of Medicine, University of Adelaide, Australia, <sup>2</sup>Satiogen Pharmaceuticals, San Diego, USA.

**Background and aims:** Glucagon like peptide-1 (GLP-1) and peptide YY (PYY) are secreted by enteroendocrine L-cells found most densely in the colon and rectum, and are of fundamental importance in blood glucose and appetite regulation. In vitro and animal studies suggest that bile salts can stimulate GLP-1 and PYY secretion by TGR5 receptor activation. We sought to evaluate the effects of rectal infusion of taurocholic acid (TCA) on the release of GLP-1 and PYY in healthy humans.

**Materials and methods:** 10 healthy males were studied after an overnight fast on 3 occasions each, in double-blind, randomised fashion. On each day, 20 mL of 1% carboxymethyl cellulose gel containing 1500 mg or 3500 mg of TCA, or vehicle only (placebo), was infused into the rectum (T = 0 min). Blood was sampled at intervals for 180 min for total GLP-1 and PYY, and gastrointestinal sensations were evaluated by 100 mm visual analogue scales. Data are mean  $\pm$  SEM. Hormone secretion was analysed as incremental area under the curve (iAUC).

**Results:** TCA enemas stimulated secretion of both GLP-1 (1500 mg TCA: 233.4  $\pm$  116.3 pmol/L  $\times$  min, 3500 mg TCA: 390.2  $\pm$  84.7 pmol/L  $\times$  min vs placebo: 61.4  $\pm$  23.4 pmol/L  $\times$  min, P = 0.019) and PYY (1500 mg TCA: 452.8  $\pm$  192.2 pmol/L  $\times$  min, 3500 mg TCA: 827.2  $\pm$  199.2 pmol/L  $\times$  min vs placebo: 4.8  $\pm$  3.2 pmol/L  $\times$  min, P = 0.0005), and the iAUC for each demonstrated a dose-dependent relationship (r = 0.48, P = 0.004 for GLP-1, and r = 0.56, P = 0.001 for PYY). Fullness was greater after 3500mg TCA than placebo (P < 0.05), without a significant difference between 1500mg TCA and placebo.

**Conclusion:** Rectal infusion of TCA stimulates GLP-1 and PYY, and increases fullness, in a dose-dependent manner in healthy humans, establishing the concept that topical application of bile acids has therapeutic potential for the management of diabetes and obesity.



Clinical Trial Registration Number: RAH: 100514

Supported by: NHMRC grant

## 821

## Safety, pharmacokinetics, and pharmacodynamics of a single subcutaneous dose of VRS-859 in patients with type 2 diabetes

J. Cleland<sup>1</sup>, R. Aronson<sup>2</sup>, E. Humphris<sup>1</sup>, C.R. Shore<sup>1</sup>, G. Bright<sup>1</sup>, R. Zhou<sup>3</sup>, M.S. Kipnes<sup>4</sup>;

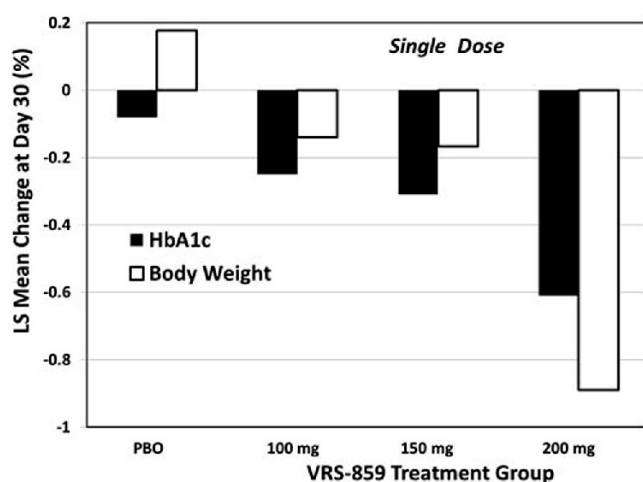
<sup>1</sup>Diartis Pharmaceuticals, Redwood City, <sup>2</sup>LMC Endocrinology Center, Toronto, Canada, <sup>3</sup>Medpace, Cincinnati, <sup>4</sup>DGD Clinic, San Antonio, USA.

**Background and aims:** VRS-859, a GLP-1 analog XTEN fusion protein, was compared to placebo in a single ascending dose (SAD) study in patients with type 2 diabetes mellitus (T2DM) using concurrent metformin monotherapy.

**Materials and methods:** The study used a randomized, multi-centre, placebo-controlled, double blind, SAD design to evaluate the safety/tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of subcutaneous (SC) VRS-859. The study group included patients with T2DM currently treated with metformin. After screening, subjects received placebo (PBO) or VRS-859 (12.5, 25, 50, 100, 150 or 200 mg) as a single SC dose. In each dosing group, 8 patients received VRS-859 and 3 patients received PBO. PK and PD measurements (fasting glucose (FG), insulin, and oral glucose tolerance test (oGTT)) were performed on Days -1, 4, 8, 11, 15, 18, 22, 25, and 30. Safety assessments including calcitonin, lipase, and amylase levels as well as QTc analyses were performed through Day 30. Anti-VRS-859 antibody analyses are performed at Day -1, Day 30 and on follow-up at Day 60. HbA<sub>1c</sub> and body weight were measured at Day -1 and Day 30.

**Results:** 70 patients (56% M) were randomized in the study (mean values: 52 y, 93 kg, 8.2% HbA<sub>1c</sub>, 171 mg/dL FG). 52 T2DM patients were treated with VRS-859 and 18 T2DM patients were treated with PBO. No serious adverse events were reported and no patients dropped out of the study. No changes in QTc interval were observed. 8 of 52 patients had a low titer (<25) anti-VRS-859 antibody response at Day 60 that was specific to exenatide. No systematic or significant individual changes in lipase, amylase, and calcitonin levels were observed through Day 30. VRS-859 was well tolerated at all dose levels including the maximum dose, 200 mg. GI adverse events were generally mild, transient, occurred prior to C<sub>max</sub> and resolved in <24 hrs. VRS-859 PK was dose linear with an average T<sub>max</sub> of 90 hr, and an average t<sub>1/2</sub> of 128 hr. VRS-859 normalized fasting glucose (<7.2 mM) in 7 of 8 patients after a single dose of 200 mg without hypoglycemia. There was a strong correlation between VRS-859 plasma concentrations and FG ( $r = -0.63$ ,  $P < 0.0001$ ). Reductions in glucose excursions post-OGTT also correlated with VRS-859 plasma concentrations. A single dose of 200 mg VRS-859 resulted in mean reductions in HbA<sub>1c</sub> and body weight of 0.61% and 0.89 kg, respectively, at Day 30. Patients with an HbA<sub>1c</sub> above 7% (n=5) in the 200 mg VRS-859 dose group had a mean HbA<sub>1c</sub> reduction of 1.06% at Day 30 after a single dose.

**Conclusion:** VRS-859 was well tolerated and provided glycemic control in T2DM patients, and monthly doses may provide sustained reductions in HbA<sub>1c</sub> and body weight.



Clinical Trial Registration Number: Eudract number: 2010-019182-29

## 822

### KRP-104, a uniquely prandial-targeted DPP4 inhibitor

D.J. Plotkin<sup>1</sup>, A. Lewin<sup>2</sup>, D. Logan<sup>3</sup>, T. Kato<sup>4</sup>, J. Kozarich<sup>1</sup>, X. Wei<sup>3</sup>, J. Vest<sup>3</sup>, D. Orloff<sup>3</sup>

<sup>1</sup>ActivX Biosciences, Inc, La Jolla, USA, <sup>2</sup>National Research Institute, Los Angeles, USA, <sup>3</sup>Medpace, Cincinnati, USA, <sup>4</sup>Kyorin Pharmaceutical Co, Ltd, Tokyo, Japan.

**Background and aims:** Dipeptidyl peptidase4 inhibitors (DPP4i) used to treat hyperglycemia exhibit differential potency depending on incretin release stimulated by glucose intake. Unlike currently available DPP4i that are continuous inhibitors of DPP4 due to their PK/PD profiles, in Phase (P)1, KRP-104 (KRP) had a PK/PD profile with a unique dose by duration response enabling either continuous or daytime, i.e. prandial-specific, DPP4 inhibition depending on dose. The aim of the KRP P1-P2 clinical develop-

ment program was to test and compare the glycemic effects of these contrasting inh profiles.

**Materials and methods:** Glycemic efficacy of continuous (Con) vs prandial (prand) only DPP4 inh was assessed in 3 studies in type 2 diabetics. P1b: 2-period open-label, balanced cross-over, 7days/period, KRP vs 100mg daily sitagliptin (sita), 14 pts/group. Group 1, KRP 80mg twice daily; Group 2, KRP 70mg daily (60mg in AM, 10mg with dinner). P2a: 12 week (wk) randomized (R), double-blind (DB), KRP 120mg once daily, 60mg twice daily, or placebo (PBO)(1:1:1) on stable metformin, n=213. P2b: 24 wk R, DB, KRP 20/120mg (dose up titrated at wk 12), 40mg, 80mg, 100mg or PBO once daily (1:1:1:1:1) on stable metformin, n=403.

**Results:** P1b KRP vs Sita. Group1: KRP at 80mg twice daily produced Con-inh of ~90-95% and glycemic efficacy similar to sita at =>80% Con-inh assessed by fasting plasma glucose (FPG), 7 point glucose (G), or Con G monitoring. No increase in glycemic effect was seen with higher inh (Table). Group2: Prand-only inhibition by KRP (70mg) achieved comparable glycemic efficacy to sita (Table). P2a Diurnal Profile Assessment: KRP 120mg once daily (prand-inh); 60mg twice daily (=>80% Con-inh). After 12 wks, there was a statistically significant change from baseline compared to PBO for both KRP groups for HbA<sub>1c</sub> and FPG, but no significant difference between KRP groups (Table). Safety parameters were comparable among all 3 groups. P2b Dose Finding. As predicted by PK/PD modeling, after 12 and 24 wks treatment, 80mg was the lowest dose that achieved the maximum HbA<sub>1c</sub> reduction (LS mean, LOCF, -0.7% 24 wk) (Table).

**Conclusion:** In all 3 studies, KRP was well tolerated with a safety profile similar to controls. KRP demonstrated similar glycemic efficacy when dosed to inhibit DPP4 continuously or only during prandial hrs, and had comparable efficacy to sita even when dosed for only prand-inh or at higher levels of Con-inh. Thus, clinical studies with KRP demonstrated that the clinically meaningful glycemic benefit of DPP4i is mediated primarily during the prandial hours and inh =>80% is sufficient for maximal effect. Thus the KRP development dose of 80 mg once daily provides a diurnal on/off inh cycle; inh DPP4 during daytime for maximal glycemic control but releases DPP4 for other functions at night (when the impact on glycemic efficacy was negligible) to potentially boost chronic safety.

### KRP-104 diurnal effects on glycemic response

#### P1b: KRP-104 vs Sitagliptin

	KRP-104 n=14	Sitagliptin n=14
Group 1: 7-point Mean Daily Glucose (mg/dL)		
Test: 90-95 vs ≥80% Con Inh	LS Mean Change from Baseline <sup>1</sup> -26.8	-30.6
Treatment comparison vs Sitagliptin	3.9 (p=0.5730)	
Group 2: FPG (mg/dL)		
Test: Con-Inh vs Prand-Inh	LS Mean Change from Baseline -11.7	-11.8
Treatment comparison vs Sitagliptin	0.1 (p=0.9797)	

#### P2a: KRP-104 120mg Once Daily (Prand-Inh) vs 60mg Twice Daily (Con-Inh)

	120mg Once Daily	60mg Twice Daily
HbA <sub>1c</sub> (%)	n 72	66
LS Mean Change vs Placebo <sup>2</sup>	-0.59 (p<0.0001)	-0.69 (p<0.0001)
FPG (mg/dL)	n 72	67
LS Mean Change vs Placebo <sup>2</sup>	-15.3 (p=0.0025)	-15.4 (p=0.0028)

#### P2b: KRP-104 20/120, 40, 80, 100mg Once Daily (Prand-Inh, Dose Finding)

	40mg	80mg	100mg	20/120mg <sup>4</sup>
HbA <sub>1c</sub> (%)	n 79	79	80	79
LS Mean Change vs Placebo <sup>3</sup>	-0.32 (p=0.0587)	-0.61 (p<0.0001)	-0.62 (p<0.0001)	-0.64 (p<0.0001)

1. LS=least squares; From linear model to analyze crossover data 2. Adjusted for baseline HbA<sub>1c</sub>

3. Adjusted for screening metformin use and baseline HbA<sub>1c</sub> 4. 20 mg for 12 wks followed by 120 mg for 12 wks

Clinical Trial Registration Number: NCT00525330, NCT00995345



## 823

**The novel GLP-1-gastrin dual agonist ZP3022 improves glycaemic control in ZDF rats**

J. Skarbaliene, J.L. Tolborg, T.S.R. Neerup, K. Fosgerau;  
Zealand Pharma A/S, Glostrup, Denmark.

**Background and aims:** Combination treatment with exendin-4 and gastrin has been shown to improve diabetes and preserve  $\beta$ -cell mass by stimulating  $\beta$ -cell growth and differentiation in diabetic mice. Here we investigated the anti-diabetic effects of a novel GLP-1-gastrin dual agonist ZP3022 in Zucker Diabetic Fatty (ZDF) rats.

**Materials and methods:** ZDF rats aged 11 weeks were dosed s.c., bid for 8 weeks with either vehicle, ZP3022 (10, 40 nmol/kg), liraglutide (40 nmol/kg), exendin-4 (30 nmol/kg), gastrin17 (80 nmol/kg), or exendin-4 + gastrin17 (30 + 80 nmol/kg). HbA1c was measured at treatment start and at termination, an oral glucose tolerance test (OGTT) was performed after 5 weeks of treatment, and non-fasting blood glucose (BG) was measured every other week.

**Results:** The changes in HbA1c levels, BG AUCs during the OGTT, and non-fasting BG at the end of the study can be seen in Table 1. ZP3022 clearly improved glycaemic control as did all treatments, except gastrin17. Notably, ZP3022 caused a significantly greater reduction in HbA1c levels than liraglutide at equimolar dose. Only ZP3022 high dose, exendin-4 and exendin-4 + gastrin17 significantly improved glucose tolerance after an OGTT. Moreover, ZP3022 BG lowering effect was already present after 2-weeks of treatment and persistent throughout the study ( $P < 0.001$  vs. vehicle). Other treatments, except gastrin17, also had a significant BG lowering effect. However, the effect of liraglutide was transient.

**Conclusion:** In conclusion, treatment with ZP3022 markedly improved glycaemic control in diabetic ZDF rats, which indicate GLP-1-gastrin dual agonism as a possible target for the treatment of diabetes.

Table 1

Treatments	$\Delta$ HbA1c (%) termination- baseline	OGTT (mM) AUC <sub>0-240 min</sub> <sup>a</sup> Y=0	Non-fasting BG(mM) termination
Vehicle	1.49 ± 0.06	5416 ± 156.7	23.33 ± 0.55
Vehicle lean	-0.25 ± 0.03 <sup>***</sup>	1288 ± 52.16 <sup>***</sup>	6.22 ± 0.16 <sup>***</sup>
ZP3022 40 nmol/kg, bid	-0.13 ± 0.1 <sup>***</sup> ^ ^	3911 ± 341.8 <sup>***</sup> ^	18.71 ± 0.34 <sup>***</sup> ^ ^ ^
ZP3022 10 nmol/kg, bid	0.57 ± 0.08 <sup>***</sup>	5292 ± 165.8	18.49 ± 0.39 <sup>***</sup>
Liraglutide 40 nmol/kg, bid	0.48 ± 0.12 <sup>***</sup>	5054 ± 160.5	22.24 ± 0.88
Exendin-4 30 nmol/kg, bid	0.34 ± 0.06 <sup>***</sup>	4649 ± 205.1 <sup>*</sup>	19.06 ± 0.46 <sup>**</sup>
Gastrin17 80 nmol/kg, bid	1.35 ± 0.11	5544 ± 149.1	24.26 ± 0.66
Exendin-4 + gastrin17 30 + 80 nmol/kg, bid	0.58 ± 0.07 <sup>***</sup>	4185 ± 162.1 <sup>***</sup>	18.98 ± 0.26 <sup>***</sup>

Data were analyzed using one-way ANOVA or Kruskal-Wallis test followed by Dunnett's multiple comparison test, <sup>\*</sup> $p < 0.05$ , <sup>\*\*</sup> $p < 0.01$ , <sup>\*\*\*</sup> $p < 0.001$  vs. vehicle; or Mann Whitney test <sup>\*\*\*</sup> $p < 0.001$  vs. vehicle, <sup>^</sup> $p < 0.05$ , <sup>^^</sup> $p < 0.01$ , <sup>^^^</sup> $p < 0.001$  vs. liraglutide. n = 9–12. Data are mean ± SEM.

## 824

**Incretin hormones in patients with type 2 diabetes are increased by diet-oil, a pro-drug for the GPR 119 receptor agonist, 2-oleoyl-glycerol**

M.J. Mandoe<sup>1</sup>, K.B. Hansen<sup>2</sup>, F.K. Knop<sup>3</sup>, J.J. Holst<sup>1</sup>, H.S. Hansen<sup>4</sup>;

<sup>1</sup>Department of Biomedical Sciences, NNF Center for Basic Metabolic Research, Copenhagen, <sup>2</sup>Department of Internal Medicine, Amager Hospital, Copenhagen, <sup>3</sup>Department of Internal Medicine, Gentofte Hospital,, <sup>4</sup>Department of Drug Design and Pharmacology, University of Copenhagen, Denmark.

**Background and aims:** Diet-oil (1,3-di-butyl-2-oleoyl-glycerol) is a precursor for the naturally occurring 2-oleoyl-glycerol (2-OG), which is a li-

gand for the G protein-coupled receptor GPR119, and causes release of the insulinotropic and glucagonostatic hormone, glucagon-like peptide-1 (GLP-1) from L cells in healthy subjects. The liberation of 2-OG from diet-oil is thought to occur more distally in the gut than 2-OG from olive oil (trioleoylglycerol). Because the density of L cells increases in the distal gut, diet-oil might generate a greater GLP-1 response than olive oil. 2-OG also stimulates release of glucose-dependent insulinotropic polypeptide (GIP).

**Materials and methods:** Diet-oil (and olive oil) were administered orally to 13 Caucasian patients with type 2 diabetes (8 males, age: 62±3 years (mean±SEM), body mass index: 30.4±2.5 kg/m<sup>2</sup>, fasting plasma glucose: 8.9±1 mmol/l, HbA<sub>1c</sub>: 6.9±0.4%) in a randomised, single-blinded cross-over study. The subjects were given the following meals on 3 different days: 200 g grated carrot, 200 g grated carrot + 20 g olive oil, or 200 g grated carrot +10.7 g diet-oil. Theoretically, the two oil regimes both result in formation of 7.7 g 2-OG during digestion. Primary outcomes were total GLP-1 and GIP measured in plasma.

**Results:** Olive oil and diet-oil resulted in significantly ( $p \leq 0.01$ ) greater post-prandial GLP-1 and GIP responses [GLP-1: incremental area under curve 845 and 710 pmol/l×180 min,  $p=0.41$ ; GIP: 4337 and 2926 pmol/l×180 min,  $p=0.08$ ] compared to the carrot meal (386 pmol/l×180 min and 874 pmol/l×180 min).

**Conclusion:** Diet-oil enhanced secretion of GLP-1 and GIP (compared to carbohydrate alone) to almost the same extent as olive oil, although olive oil liberates not only 2-OG, but in addition two oleic acid molecules, which may also stimulate incretin secretion. Thus, diet oil is more effective as incretin releaser than olive oil per unit of energy content and could be useful for dietary intervention.

Clinical Trial Registration Number: NCT01453842

Supported by: UNIK. Food, Fitness and Pharma.

## 825

**Pharmacokinetics and pharmacodynamics of a new exenatide formulation, exenatide suspension**

L. Porter, S. Flore, B. Cirincione, M. Zierhut, C. Biwald, W. Huang, T. Booker Porter, L. MacConell;

Amylin Pharmaceuticals, Inc., San Diego, USA.

**Background and aims:** Exenatide, a GLP-1 receptor agonist, has been shown to improve glycaemic control and body weight with twice-daily or once-weekly subcutaneous injections in patients with T2DM. Exenatide suspension uses the exact same drug substance and drug load (5% by mass) in extended-release microspheres as exenatide once weekly (EQW) but is reformulated with a medium chain triglyceride (MCT) vehicle that has a history of safe use in humans. This formulation was developed to 1) yield a presuspended product not requiring reconstitution of microspheres just prior to injection as with EQW, 2) be compatible with an easier-to-use pen device, 3) further reduce the initial exenatide release that appears to affect gastrointestinal tolerability.

**Materials and methods:** An in vitro assay performed at 37°C was used to compare the profiles of exenatide release from the microspheres when suspended in the different vehicles used for EQW and exenatide suspension. The pharmacokinetics (PK), safety, and efficacy of exenatide suspension were assessed in a Phase 2, two-cohort study. Subjects in Cohort 1 (30 healthy volunteers) received a single 10mg dose of exenatide suspension; subjects in Cohort 2 (35 subjects with T2DM (31% female, 52±11 y, WT 105±22 kg, HbA1c 8.0±0.9%, FPG 9.28±1.93 mmol/L, [mean±SD] treated with diet/exercise, metformin [MET], pioglitazone [PIO] or MET+PIO) were randomized to exenatide once weekly suspension (EQWS, 2 mg, N=23) or MCT vehicle control (N=12) for 12 weeks, with primary analyses performed at week 12.

**Results:** Using the in vitro method to compare release profiles, exenatide suspension demonstrated a blunted initial release compared to EQW but a similar overall release profile. Administration of a single 10-mg dose in healthy volunteers confirmed the in vitro profile; exenatide concentrations increased gradually over time, peaked at week 6–7, and approached lower level of detection after ~week 10. While initial early release was reduced relative to EQW (as observed during the first day post-dose), the extent and duration of exposure following a single dose of EQWS was similar to that of EQW. Nonparametric superpositioning simulations of the singledose data predicted similar steady-state concentrations (C<sub>ss</sub>) with 2 mg EQWS compared to 2 mg EQW. These simulations were confirmed with Cohort 2, where EQWS achieved mean C<sub>ss</sub> by ~week 8 that were in the range of C<sub>ss</sub> previously seen with EQW. At week 12, the LS mean (SE) change from baseline was significantly greater with EQWS than MCT for HbA1c (0.9 [0.2] vs. +0.1 [0.2]%,  $P=0.0013$ ) and

FPG (1.78 [0.55] vs. +0.44 [0.67] mmol/L,  $P=0.0035$ ) and was associated with weight loss (-1.4 [0.6] vs. -0.4 [0.7] kg,  $P=0.2285$ ). No unique safety findings were observed with EQWS relative to EQW.

**Conclusion:** EQWS was well tolerated, with improvements in glycaemic control and weight loss in patients with T2DM comparable to EQW. The similarities in the overall PK, safety, and efficacy profiles of EQWS compared to EQW confirm the appropriateness of the 2-mg weekly dose. These findings support the further development of this once-weekly presuspended product as a formulation that has the opportunity to improve the overall patient experience with their course of treatment by simplifying the drug preparation and administration process.

Clinical Trial Registration Number: NCT00894322

## 826

### Safety, tolerability, pharmacokinetics (PK) / pharmacodynamics (PD) of single escalating doses of semaglutide, a unique once weekly GLP-1 analogue, in healthy male subjects

C. Kapitza<sup>1</sup>, J. Lyng<sup>2</sup>, M. Düring<sup>2</sup>, C. Jensen<sup>2</sup>;

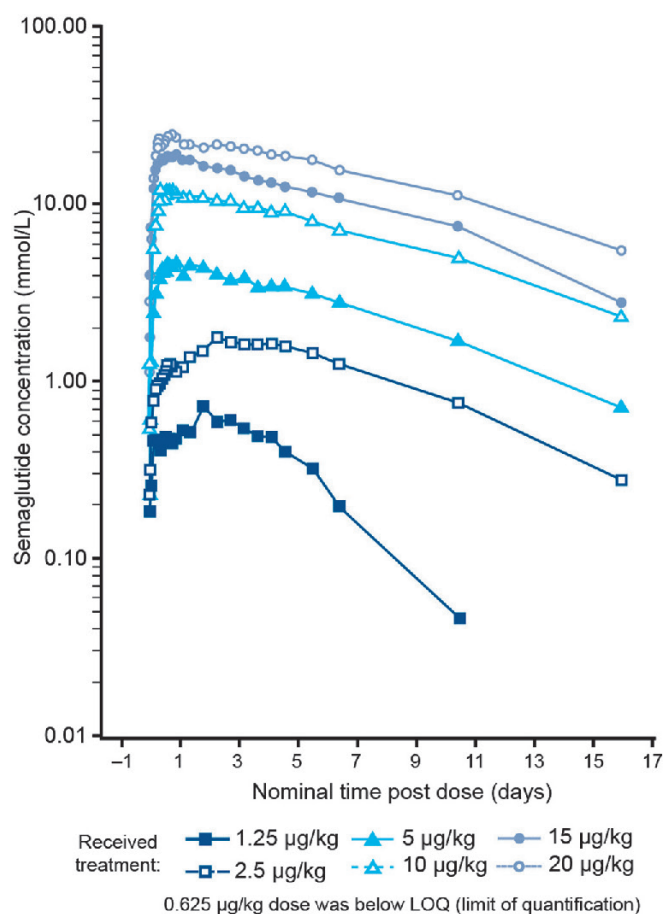
<sup>1</sup>Profil Institut, Neuss, Germany, <sup>2</sup>Novo Nordisk, Søborg, Denmark.

**Background and aims:** Medications administered once-weekly have the potential to improve patient compliance and thereby treatment outcomes. The aim of this first-in-man trial was to investigate safety, tolerability, PK and PD of the acylated, human GLP-1 analogue, semaglutide, aiming to establish the maximum tolerated single dose (MTSD).

**Materials and methods:** This was a randomised, double-blind, placebo-controlled, single-dose, dose escalation trial in healthy males. We randomised 56 subjects (active 6; placebo 2). Semaglutide doses: 0.625, 1.25, 2.5, 5, 10, 15 or 20 µg/kg. PK was assessed up to 19 days post-dose. Dosing day is Day 1. PD assessments included fasting plasma glucose (FPG), fasting insulin and glucagon (pre-dose, Day 2, Day 8), 14-h profiles of glucose, insulin and glucagon (Day 2), appetite ratings (Visual Analogue Scores, VAS) during a standard lunch meal, and energy intake (EI) during an *ad lib* dinner meal (pre-dose, Day 2, Day 8), and body weight (pre-dose and at multiple time points post-dose). MTSD was the dose at which less than 50% of subjects in a cohort had moderate or severe adverse events (AEs) related to trial product.

**Results:** MTSD was 15 µg/kg body weight (1.2 mg in an 80 kg subject). There were no serious AEs; all AEs were mild or moderate, and no subjects were withdrawn due to AEs. Most common AEs were nausea, dyspepsia and vomiting (10, 15, 20 µg/kg doses), headache, and decreased appetite (15, 20 µg/kg doses only). No treatment effect was observed on safety lab parameters (including calcitonin), vital signs, physical examination and ECG. No hypoglycaemia was reported. Plasma mean elimination half-life was 155–173 h (10, 15, 20 µg/kg doses). Median  $t_{max}$  was 16–20 h (10, 15, 20 µg/kg doses). Dose proportionality was shown for  $AUC_{0-48h}$ ,  $AUC_{0-168h}$ ,  $AUC_{0-inf}$  and  $C_{max}$  (10, 15, 20 µg/kg doses). There was an overall treatment effect on FPG ( $p=0.013$ ), fasting insulin ( $p<0.001$ ) and fasting glucagon ( $p=0.04$ ) on Day 2, and on fasting glucagon on Day 8 ( $p=0.011$ ). Further, there was an overall treatment effect on  $AUC_{0-14h \text{ post-dose}}$  for these parameters on Day 2 ( $p<0.001$ ,  $p<0.001$ ,  $p=0.038$ ). Mean VAS profiles indicated reduced hunger and prospective food consumption, and increased fullness and satiety, during the standard lunch meal at doses  $\geq 10$  µg/kg compared to placebo on Day 2, but not on Day 8 (statistical testing not done). Mean energy intake during the *ad lib* dinner meal on Days 2 and 8 appeared reduced at doses  $\geq 10$  µg/kg compared to placebo (statistical testing not done). Mean body weight decreased from baseline to follow-up (4 weeks post-dose) in most groups, incl. placebo, ranging from 0.9 kg with placebo to 1.2–2.8 kg for doses  $\geq 5$  µg/kg.

**Conclusion:** No safety concerns were identified; single s.c. doses of semaglutide were safe and well tolerated, and the PK profile was compatible with once-weekly administration. The observed effects on appetite, food intake and body weight may translate into significant clinical benefits with chronic treatment.



Clinical Trial Registration Number: EudraCT 2007-000303-15

Supported by: Novo Nordisk

## PS 065 DPP-4 and other secretagogues

827

### Consistency of HbA<sub>1c</sub>-lowering effects of sitagliptin vs glipizide in patients with type 2 diabetes and chronic renal insufficiency across a variety of baseline characteristics

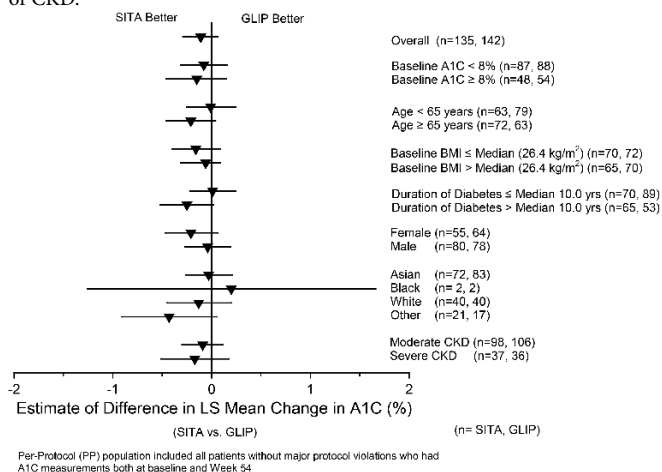
S. Engel, J.C. Arjona Ferreira, H. Guo, G. Golm, A.O. Johnson-Levonas, K. Kaufman, B.J. Goldstein; Merck Sharp & Dohme, Whitehouse Station, USA.

**Background/Synopsis:** In a 54-week, randomized, double-blind study conducted in patients with type 2 diabetes mellitus (T2DM) (n=423) with moderate or severe chronic kidney disease (CKD) (estimated glomerular filtration rate 30 to <50 or <30 mL/min/1.73 m<sup>2</sup>, respectively) sitagliptin (SITA) was previously shown to be non-inferior to glipizide (GLIP) in lowering A1C in the prespecified per protocol (PP) population (n=277).

**Methods:** Eligible patients for the study were ≥30 years of age with T2DM and moderate to severe CKD not on dialysis with A1C ≥7.0 and ≤9.0% on diet/exercise alone. Patients were randomized (1:1) to SITA or GLIP, and randomization was stratified by CKD status (moderate or severe). Doses of SITA for patients with moderate and severe CKD were 50 mg/d and 25 mg/d, respectively; the dose was adjusted downward (from 50 to 25 mg/d) if CKD status changed from moderate to severe during the study. The initial GLIP dose was 2.5 mg/d and could be titrated up to 10 mg BID as needed for glycemic control; the dose could also be reduced or interrupted to prevent hypoglycemia. The present analysis assessed the consistency of the A1C-lowering effects of SITA vs GLIP across prespecified subgroups of the PP population (baseline A1C, age, body mass index, diabetes duration, gender, race and severity of CKD).

**Results:** For the overall PP population, least squares (LS) mean changes from baseline in A1C were -0.76% (SITA) and -0.64% (GLIP), with a between-group difference (95% CI) of -0.11% (-0.29, 0.06). The between-group differences were generally consistent across the prespecified subgroups (Figure).

**Conclusion:** In patients with T2DM and CKD, treatment with SITA produced A1C reductions similar to those observed with GLIP, irrespective of baseline A1C, age, body mass index, diabetes duration, gender, race or severity of CKD.



Clinical Trial Registration Number: NCT00509262

Supported by: Merck Sharp & Dohme Corp.

828

### Islet-independent mechanisms contribute to glucose-lowering effect of sitagliptin after mixed meal and oral glucose in non-diabetic subjects

L. Ohlsson<sup>1</sup>, W. Alsalm<sup>1</sup>, R.D. Carr<sup>2</sup>, G. Pacini<sup>3</sup>, A. Mari<sup>4</sup>, B. Åhrén<sup>1</sup>; <sup>1</sup>Dept of Clinical Sciences, Lund, Sweden, <sup>2</sup>Merck A/S, Glostrup, Denmark, <sup>3</sup>ISIB-CNR Corso Stati Uniti, Biomedical Engineering, Padova, Italy, <sup>4</sup>Biomedical Engineering, Padua, Italy.

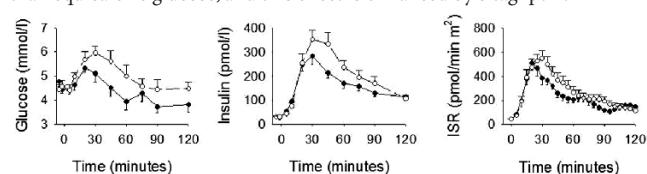
**Background and aims:** DPP-4 inhibitors improve glycemia by increasing the levels of glucagon-like peptide-1 (GLP-1) which stimulates insulin secretion and inhibits glucagon secretion at elevated glucose levels. We explored whether DPP-4 inhibition affects islet function at physiological glucose levels, and if DPP-4 inhibition differently affects islet GLP-1 and islet hormones after intake of mixed meal, oral glucose and oral lipid.

**Materials and methods:** Twelve healthy non-obese males ingested either a standardized mixed meal (560 kcal), glucose (2g/kg) or fat (olive oil; 0.9g/kg) together with DPP-4 inhibitor sitagliptin (100mg) or placebo. Blood samples were taken for analyses of glucose, intact GLP-1, insulin, C-peptide and glucagon; C-peptide deconvolution was used to estimate insulin secretory rate (ISR). Paracetamol test was used for determining gastric emptying. Suprabasal AUC were estimated.

**Results:** All three challenges increased plasma intact GLP-1 levels with augmented effects of sitagliptin. Fat was most potent in increasing GLP-1. Thus, the 120 min AUC being 863±123 (fat), 572±129 (glucose) and 220±80 pmol/l x min (meal) after placebo, increased to 2593±466 (fat), 1324±237 (glucose) and 502±113 pmol/l x min (meal) by sitagliptin (P<0.005 for all challenges). Fig. 1 shows that sitagliptin almost completely suppressed the raise in glucose after meal (AUC 46±16 vs 70±15 mmol/l x min; P=0.03). This was associated with reduced insulin levels (AUC 16±1 nmol/l x min vs 21±2 nmol/l x min P=0.02). ISR (20-60 min) was lower with sitagliptin than placebo (AUC 11±1 vs 15±2 nmol/min, m2, p=0.02), although sitagliptin increased ISR when compared at similar glucose levels. Also after glucose, sitagliptin prevented the increase in glucose with a reduction of insulin levels and ISR, similarly as after meal. After fat, glucose levels were reduced, insulin and ISR were not changed, and no additional effect of sitagliptin. Glucagon levels were increased after meal, reduced after glucose and not affected after fat, without statistically significant effects of sitagliptin. Gastric emptying was not altered by sitagliptin.

**Conclusion:** The increased glycemia after meal and glucose is prevented by sitagliptin without preceding increase in insulin, ISR, or reduction in glucagon in non-diabetic subjects, yet, when compared at identical glucose levels, insulin secretion is stimulated by sitagliptin. This suggests that extra-islet effects contribute to the glucose-lowering effect by sitagliptin, that an insulino-tropic effect of sitagliptin is manifested at as low glucose levels as 5 mmol/l. Also, the high GLP-1 after sitagliptin and fat does not affect islet hormone secretion at low glucose levels. Finally, fat is more powerful to raise GLP-1 than equicaloric glucose, and this effect is enhanced by sitagliptin.

**Conclusion:** The increased glycemia after meal and glucose is prevented by sitagliptin without preceding increase in insulin, ISR, or reduction in glucagon in non-diabetic subjects, yet, when compared at identical glucose levels, insulin secretion is stimulated by sitagliptin. This suggests that extra-islet effects contribute to the glucose-lowering effect by sitagliptin, that an insulino-tropic effect of sitagliptin is manifested at as low glucose levels as 5 mmol/l. Also, the high GLP-1 after sitagliptin and fat does not affect islet hormone secretion at low glucose levels. Finally, fat is more powerful to raise GLP-1 than equicaloric glucose, and this effect is enhanced by sitagliptin.



**Figure 1.** Plasma glucose and insulin levels and insulin secretory rate (ISR) calculated by deconvolution from C-peptide data after ingestion of mixed meal in non-diabetic healthy subjects with concomitant administration of sitagliptin (●-●) or placebo (○-○). Means±SEM are shown.

Clinical Trial Registration Number: 2009/106

Supported by: VR, Region Skane, Medical Faculty, Merck

829

### Sitagliptin added to previously taken anti-diabetic agents on insulin-resistance and lipid profile: a two years study evaluation

P.D. Ragonesi<sup>1</sup>, P. Maffioli<sup>2</sup>, A.F.G. Cicero<sup>3</sup>, M.A. Ferraro<sup>4</sup>, A. Bonaventura<sup>2</sup>, L. Bianchi<sup>2</sup>, D. Romano<sup>2</sup>, E. Fogari<sup>2</sup>, G. Derosa<sup>2</sup>;

<sup>1</sup>Diabetes Care Unit, S. Carlo Hospital, Milano, Italy, <sup>2</sup>Internal Medicine and Therapeutics, University of Pavia, IRCCS Policlinico S. Matteo, Italy,

<sup>3</sup>"G. Descovich" Atherosclerosis Study Center, University of Bologna, Italy,

<sup>4</sup>Diabetologia, PST Gallico, Reggio Calabria, Italy.

**Background and aims:** Sitagliptin was the first DPP-4 inhibitor to be released for the use in the clinical practice; it is actually licensed at the recommended dose of 100 mg once daily either as monotherapy or in combination for the treatment of type diabetes mellitus in patients who have an inadequate glycaemic control. Sitagliptin already proved to be safe and effective in relatively short term studies, giving also a greater durability of β-cell compared to sulfonylureas. The aim of this study is to evaluate if the positive effects of sitagliptin on glycaemic control, insulin resistance and β-cell function are maintained also after 2 years of therapy, and if sitagliptin can be effective also in improving lipid profile.

**Materials and methods:** In this randomised, double-blind, placebo-controlled trial 205 type 2 diabetic patients in therapy with different anti-diabetic



drugs were randomised to add sitagliptin 100 mg once a day or placebo to their current therapy for 24 months. We evaluated at the baseline and after 6, 12, 18, and 24 months: body mass index (BMI), glycated hemoglobin (HbA<sub>1c</sub>), fasting plasma glucose (FPG), post-prandial glucose (PPG), fasting plasma insulin (FPI), homeostasis model assessment insulin resistance index (HOMA-IR), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), triglycerides (Tg). Continuous variables were compared by analysis of variance (ANOVA). Intervention effects were adjusted for additional potential confounders using analysis of covariance (ANCOVA). ANOVA was also used to assess the significance within and between groups. The statistical significance of the independent effects of treatments on the other variables was determined using ANCOVA. A 1-sample *t* test was used to compare values obtained before and after treatment administration; 2-sample *t* tests were used for between-group comparisons. For all statistical analyses, *p*<0.05 was considered statistically significant. **Results:** Sitagliptin, added to previously taken anti-diabetic agents, proved to be more effective in improving glycaemic profile, reducing HbA<sub>1c</sub> of -13.2% (*p*<0.01), FPG of -12.7% (*p*<0.05), PPG of -20.5% (*p*<0.01) compared to placebo. Regarding insulin resistance, sitagliptin decreased FPI of a -8.3% (*p*<0.05 vs placebo) and HOMA-IR of a -20.0% (*p*<0.01 vs placebo), confirming that what have been already reported in short term studies can be applied also after two years of treatment. Sitagliptin also reduced body weight of -4.3% (*p*<0.05 vs placebo). Our study also showed the positive effect of sitagliptin on lipid profile, in particular, sitagliptin decreased TC of a -13.3%, LDL-C of -20.4%, and Tg of -32.3%, and also increased HDL-C of +13.6% (*p*<0.05 vs placebo for all).

**Conclusion:** Sitagliptin proved to be effective on glycaemic profile, and insulin resistance even after 2 years of therapy, and to be effective in improving body weight, and lipid profile.

## 830

### Sitagliptin vs saxagliptin in decompensated type 2 diabetes mellitus patients

A. Asti, A. D'Alessandro, G. D'Alessandro, G. Fertuso, G. Perrone, S. Nardi, G. Maresca, P. Bellis; Loreto Mare Hospital, Naples, Italy.

**Background and aims:** Sitagliptin and saxagliptin are oral hypoglycemic agents inhibitors of DPP-4, indicated in the treatment of type 2 diabetes mellitus in combination with metformin, thiazolidinediones, sulfonylureas and insulin, in patients who have not achieved adequate glycemic control.

**Materials and methods:** In our study we enrolled 128 patients (67 F and 61 M), mean age 64.3 years (SD +/- 8.4), with an average duration of diabetes of 6.5 years (SD +/- 4.5), who, already taking metformin maximum dosage, presented themselves to our observation with glycated hemoglobin > 7.5% and fasting blood glucose > 140 mg / dl. At time 0' we have detected, moreover, weight, BMI, total cholesterol, HDL and LDL, triglycerides, transaminases and pancreatic amylase; patients were randomized to receive sitagliptin 100 mg/day (64 pts., 33 F and 31 M) or saxagliptin 5 mg/day (64 pts., 34 F and 30 M); follow-up was performed after 4 months with the revaluation of the same variables and adverse events.

**Results:** In sitagliptin group we observed a reduction in fasting glucose from 187 mg/dl to 138 mg/dl, glycated hemoglobin from 8.2% to 7.1%, weighing from 88 kg to 85.4 kg, BMI from 32.2 to 31, triglycerides from 192 mg/dl to 156 mg/dl; these reductions were statistically significant, while the reduction in total cholesterol and LDL cholesterol and a slight increase in HDL cholesterol did not reach statistical significance, there were no changes in pancreatic amylase. In saxagliptin group we observed a reduction in fasting glucose from 191 mg/dl to 160 mg/dl, glycated hemoglobin from 8.1% to 7.5%, body weight from 86.5 kg to 84.4 kg, BMI from 28 to 27.5, triglycerides from 178 mg/dl to 154 mg/dl, with statistical significance; also in this group the reduction of total cholesterol, LDL cholesterol did not reach statistical significance, HDL cholesterol appeared virtually unchanged, as well as pancreatic amylase. There were no suspension of therapy, adverse events appeared minor and temporary: in sitagliptin group we observed three cases of nausea and one case of nasopharyngitis, which resolved after ten days, in the saxagliptin group two cases of nausea and one case of headache which resolved quickly.

**Conclusion:** In conclusion, our observations highlight the almost identical efficacy of sitagliptin and saxagliptin in reducing blood glucose and glycated hemoglobin, with a favorable impact on weight and plasma lipids, in the presence of insignificant side effects: these data reinforce even more the idea that should think about this class of drugs as the next step in patients failing therapy with metformin.

## 831

### Combination therapy with low dose glimepiride and sitagliptin is effective for poorly controlled Japanese patients with type 2 diabetes

H. Ishii, M. Takei, S. Nisio, Y. Sato, S. Suzuki, M. Komatsu; Department Aging Medicine and Geriatrics, Shinshu University School of Medicine, Matsumoto, Japan.

**Background and aims:** The committee of Japan Association for Diabetes Education and Care has recommended that the dose of glimepiride should be decreased to at least 2 mg/day in case of combination therapy of sitagliptin and glimepiride to patients with higher doses of glimepiride in order to prevent unexpected hypoglycemia. Based on our preliminary clinical experiences, we suppose that the dose of glimepiride could be decreased to 1 mg/day without reducing its efficacy given 50 mg/day sitagliptin is added.

**Materials and methods:** To assess the efficacy and safety of the combination therapy with low dose glimepiride and sitagliptin for poorly controlled type 2 diabetes patients, we studied 34 patients with poorly controlled type 2 diabetes who are taking high dose of glimepiride at several hospitals or clinics in Nagano Prefecture, Japan. We decreased the dose of glimepiride to 1 mg/day and added 50mg/day of sitagliptin in all 34 patients. We divided 34 patients into three groups, the first one was low dose group (the dose of glimepiride was decreased from 2mg to 1mg), the second was moderate dose group (the dose of glimepiride was decreased from 3mg or 4mg to 1mg) and the third was high dose group (the dose of glimepiride was decreased from 6 mg to 1 mg). Other oral hypoglycemic agents except sulfonylureas were continued. We monthly checked HbA<sub>1c</sub>, casual plasma glucose, 1,5-AG, body weight and subjective symptoms in each group for 6 months.

**Results:** The mean age of patients was 68.2 years old and body mass index (BMI) was 25.8 kg/cm<sup>2</sup>. The average daily dose of glimepiride was 3.9mg. HbA<sub>1c</sub> was efficiently decreased during study period with combination therapy. (Before study; 8.3±0.6%, 4weeks; 7.7±0.5, 12weeks; 7.0±0.5, 24weeks; 7.0±0.5%). The average rate of decrease of HbA<sub>1c</sub> was 1.3% in low dose glimepiride group, 1.5% in moderate dose group and 1.2% in high dose group. Casual plasma glucose, 1,5-AG, BMI have not showed statistically significant changes. The combination therapy was well tolerated except that two patients temporarily had mild abdominal symptoms. Any hypoglycemic episodes did not occur during study period. The some of the patients (n=8) were followed up for a year without apparent deterioration of HbA<sub>1c</sub> (Figure 1).

**Conclusion:** In combination therapy with glimepiride and sitagliptin, reducing the dose of glimepiride to 1mg/day from any dose has showed a sufficient antihyperglycemic effect. This efficacy has lasted for at least 1 year. In the case of the combination therapy with glimepiride and sitagliptin for poorly controlled Japanese type 2 diabetes patients, we propose that it would be appropriate to reduce the dose of glimepiride to 1mg/day from the aspect of both the efficacy and the safety.

### Change of HbA<sub>1c</sub> for 12 months

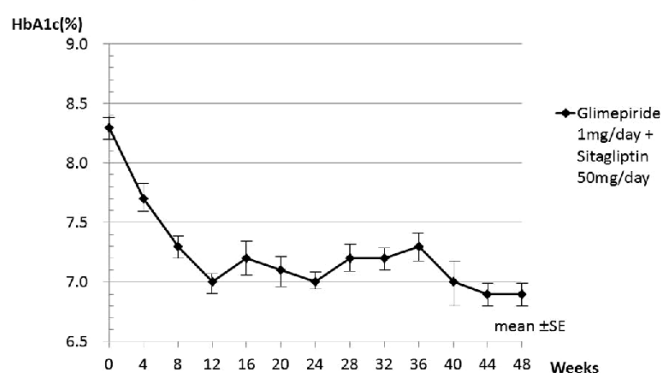


Figure 1

## 832

# Efficacy and safety of exenatide once weekly vs sitagliptin in patients with type 2 diabetes mellitus: a post hoc analysis of pooled data from DURATION-2 and -4 clinical trials

K. Herrmann, J. Han, A. Meloni, M. Wintle, J. Malloy;  
Amylin Pharmaceuticals, Inc., San Diego, USA.

**Background and aims:** Therapies that leverage the glucagon-like peptide-1 (GLP-1) pathway are generally not associated with hypoglycaemia or weight gain and have been recommended for use in type 2 diabetes mellitus patients unable to achieve glycaemic control. This post hoc analysis compared the effects of 2 GLP-1-based therapies, exenatide once weekly (EQW), a GLP-1 receptor agonist, and sitagliptin (Sita), a dipeptidyl peptidase-4 inhibitor, on glucose control, body weight, tolerability, and safety.

**Materials and methods:** Data from DURATION-2 and -4 randomized, controlled, double-blind, clinical trials examining EQW and Sita on background therapies of diet and exercise and/or metformin for 26 weeks, were pooled and stratified by baseline HbA1c. Data were analyzed using the analysis of covariance (ANCOVA) model with the last observation carried forward (LOCF) method for missing data imputation.

**Results:** Baseline characteristics were 45% female, and mean age 52 years, weight 88 kg, and HbA1c 8.5%. Changes in HbA1c, fasting plasma glucose (FPG), and body weight, and the incidence of adverse events (AEs) including hypoglycaemia were examined. Significantly greater reductions in HbA1c and FPG occurred with EQW compared to Sita across all baseline strata of HbA1c, and significantly more patients in the EQW group attained the HbA1c targets of <7% and ≤6.5% compared to the Sita group (Table). Patients treated with EQW lost significantly more body weight than patients treated with Sita (LS mean [±SE] -2.16 [0.18] kg EQW; -0.84 [0.20] kg Sita;  $P<0.0001$ ). No major hypoglycaemia and a low incidence of minor hypoglycaemia were seen in both groups (1.7% EQW; 1.5% Sita). Serious AEs were observed with similar incidence in both groups (2.2% EQW; 2.4% Sita) and more AEs led to withdrawal in the EQW group compared to the Sita group (4.2% EQW; 1.8% Sita). The incidences of the most frequent AEs of nausea (18.6% EQW; 6.7% Sita) and diarrhea (14.7% EQW; 7.6% Sita) were higher in EQW compared to Sita patients. Incidence of these AEs decreased over time to reach similar levels by study end, with nausea (0.3% EQW; 0.0% Sita) and diarrhea (0.3% EQW; 0.7% Sita) having similar incidences by >24 to 26 weeks of treatment. A similarly low percent of patients discontinued due to nausea (0.5% EQW; 0.3% Sita) or diarrhea (0.7% EQW; 0.6% Sita).

**Conclusion:** In summary, significantly greater improvements in HbA1c, FPG, and body weight were observed in patients treated with EQW compared to Sita, regardless of baseline glycaemic control. No major hypoglycaemia and equally low rates of minor hypoglycaemia were observed across the treatment groups. EQW and Sita were both generally well tolerated.

Efficacy Parameter	Baseline HbA1c subpopulations					
	≤6.5% to ≤7.5%		>7.5% to ≤9%		>9%	
	EQW (n=113)	SITA (n=91)	EQW (n=172)	SITA (n=128)	EQW (n=123)	SITA (n=108)
Baseline HbA1c, %	7.22 (0.23)	7.19 (0.25)	8.24 (0.44)	8.21 (0.44)	10.02 (0.70)	9.95 (0.64)
Δ HbA1c from baseline	-0.69 (0.07)	-0.36 (0.08)	-1.33 (0.08)	-0.86 (0.09)	-2.29 (0.13)	-1.67 (0.14)
Treatment difference	-0.33 (0.10)**		-0.47 (0.12)**		-0.61 (0.19)**	
% at HbA1c ≤6.5% at endpoint	61**	32	49**	19	24*	10
% at HbA1c <7% at endpoint	79*	62	66**	38	37**	16
Baseline FPG, mmol/L	7.69 (1.32)	7.76 (1.31)	9.25 (2.39)	9.25 (2.28)	11.79 (3.16)	11.14 (2.85)
Δ FPG from baseline	-1.15 (0.13)	-0.49 (0.15)	-1.98 (0.16)	-1.06 (0.19)	-3.18 (0.29)	-1.78 (0.31)
Treatment difference	-0.67 (0.20)**		-0.92 (0.25)**		-1.40 (0.42)**	

\* $P<0.05$ , \*\* $P<0.005$ ;  $P$  value compares EQW with Sita.

Baseline data are mean (±SD); Δ from baseline and treatment difference data are LS mean (±SE).

Intent-to-treat population: EQW N=408; Sita N=329; n=2 patients had HbA1c <6.5% at baseline in the Sita group and were not included in the HbA1c subpopulations.

Clinical Trial Registration Number: NCT00637273; NCT00676338

## 833

# Optimisation of glycaemic control with the addition of sitagliptin in type 2 diabetic patients inadequately controlled with insulin

A. Marco, A. Luque, A. Vicente, I. Luque, E. Castro, B. Cánovas, J. Sastre, E. Maqueda, V. Peña, O. Llamazares, J. López;  
Department of Endocrinology and Nutrition, Complejo Hospitalario Toledo, Spain.

**Background and aims:** Several clinical trials have shown a reduction in HbA1c when DPP-IV inhibitors are added to insulin therapy in patients with type 2 diabetes.

**Objectives:** To evaluate the efficacy of sitagliptin when added to different regimens of insulin therapy in patients with type 2 diabetes.

**Materials and methods:** A total of 46 type 2 diabetic patients inadequately controlled (HbA1c >7%) with insulin (alone or in combination with other oral antidiabetic agents), were studied prospectively during 24 weeks once sitagliptin was added. Data regarding demographic characteristics, cardiovascular risk factors, diabetic complications and metabolic control parameters were collected. Comparison of categorical variables was performed using the chi-square test, paired t-test was used for comparison of continuous variables prior and after sitagliptin addition. Comparative differences were considered as statistically significant when  $p<0.05$  (SPSS v 16).

**Results:** 41,3% of the 46 patients studied were men, mean age 66,4±10,7 years, mean diabetes duration 17,3±11,8 years. 41,3 % received basal insulin, 47,8% premixed insulin, 4,3% bolus-basal and 6,5% basal plus regimen, 80,4% received metformin, 26,1% sulfonylureas, 13% repaglinide. 47,8% had retinopathy, 26,1% nephropathy, 30,4% polyneuropathy, 15,2% ischemic heart disease, 8,7% cerebrovascular disease, 8,7% peripheral vascular disease. 67,4% obesity 58,7% arterial hypertension, 65,2% hyperlipidemia, 69,6% antiagregant therapy. The addition of sitagliptin reduced significantly HbA1c from 8,3±0,8 to 7,3±0,9% ( $p<0.001$ ) and fasting plasma glucose (FPG) from 169,8±47,1 to 135,2±42,5 mg/dl ( $p<0.001$ ). When assessed by initial HbA1c stratum (<8%, 8–9%, >9%) the HbA1c reduction was 0,6%, 1,2% and 2,0% respectively. The proportion of patients with HbA1c <7% was 44,4% of the entire cohort (57,9%, 35% and 33% in each stratified group). The HbA1c response was similar in all insulin regimens and no differences were found considering the duration of the diabetes. There was no significant change from baseline in body weight (80,9±13,2 to 80,8±12,7), total daily insulin dose/body weight (0,51±0,3 to 0,52±0,3 UI/kg), systolic pressure (142,4±20,7 to 139,0±16 mmHg), diastolic pressure (76,9±12,3 to 76,3±9,5 mmHg) and lipid parameters (LDL cholesterol 94,23±31,2 to 81,53±18,4 mg/dl).

**Conclusion:** The addition of sitagliptin to ongoing insulin therapy provided significant reduction in HbA1c without weight gain after 24 weeks of treatment and enabled a great proportion of patients to achieve the HbA1c target, even in patients with a long diabetes duration.

## 834

# Sitagliptin provides similar glycaemic improvement with less hypoglycaemia in the elderly with type 2 diabetes mellitus compared to sulphonylurea

R. Shankar, S.S. Engel, L. Xu, G.T. Golm, M.J. Davies, K.D. Kaufman, B.J. Goldstein;  
Merck Sharp & Dohme, Whitehouse Station, USA.

**Background and aims:** The global burden of type 2 diabetes mellitus (T2DM) in the elderly (≥65 years), who present unique therapeutic challenges due to comorbidities, is estimated to increase by 134% in 2030 compared to 2000. Sulphonylurea use is associated with greater risk for hypoglycaemia in the elderly and its use increases with age. Hypoglycaemia and its consequences may be more pronounced in the elderly. Sitagliptin, a DPP-4 inhibitor, improves glycaemic control, with a low risk of hypoglycaemia when used alone or with metformin. The present *post hoc* analyses compared the efficacy and safety of sitagliptin versus sulphonylurea in elderly patients with T2DM.

**Methods:** The data of patients ≥65 years of age were pooled from 3 double-blind studies to compare the effects of sitagliptin (100 mg/day) or sulphonylurea (in titrated doses) on change from baseline in HbA<sub>1c</sub>, fasting plasma glucose (FPG), and body weight and incidence of symptomatic hypoglycaemia. Patients on diet alone or metformin were randomized to sitagliptin or glipizide for 104 weeks (Studies 1-2) or glimepiride for 30 weeks (Study 3); hence, the analyses included 373 elderly patients who completed trials through 30 weeks.

**Results:** Both HbA<sub>1c</sub> and FPG decreased with sitagliptin and sulphonylurea, with no statistical difference between treatments (Table). The proportion of

patients with an  $\text{HbA}_{1c} < 6.5\%$  was similar between treatments. Significantly lower incidence of symptomatic hypoglycaemia was observed with sitagliptin relative to sulphonylurea. Body weight decreased significantly from baseline with sitagliptin. Significantly more patients on sitagliptin than sulphonylurea achieved the composite endpoint of  $>0.5\%$   $\text{HbA}_{1c}$  reduction with no hypoglycaemia or body weight gain at 30 weeks.

**Conclusion:** Sitagliptin provided similar glycaemic efficacy, with less hypoglycaemia and with body weight loss compared to sulphonylurea in elderly patients, suggesting that sitagliptin is an effective and well-tolerated treatment option for elderly patients with T2DM.

	Sitagliptin N = 178	Sulphonylureas N = 195
Baseline $\text{HbA}_{1c}$ , %	$7.5 \pm 0.7$	$7.5 \pm 0.8$
$\Delta \text{HbA}_{1c}$ , %	$-0.73 (-0.84, -0.61)$	$-0.78 (-0.89, -0.67)$
$\text{HbA}_{1c} < 6.5\%$ , n (%)	63 (35.4)	73 (37.4)
Baseline FPG, mmol/L	$8.4 \pm 1.8$	$8.7 \pm 2.1$
$\Delta \text{FPG}$ , mmol/L	$-1.2 (-1.5, -0.9)$	$-1.3 (-1.6, -1.0)$
Patients with HYPO AE, n (%)	11 (6.2)	55 (28.2)*
Baseline BW, kg	$84.6 \pm 14.6$	$83.6 \pm 15.1$
$\Delta \text{BW}$ , kg	$-1.7 (-2.3, -1.2)$	$0.4 (-0.1, 1.0)^*$
Composite <sup>†</sup> , n (%)	78 (44.1)	31 (15.9)*

AE = adverse event

Data are mean  $\pm$  SD, LS mean change ( $\Delta$ ) from baseline (95% CI), or counts (proportion of patients).

\* $p < 0.001$  for difference between sitagliptin and sulphonylurea.

<sup>†</sup>Composite = patient experienced an  $\text{HbA}_{1c}$  decrease  $>0.5\%$  with no symptomatic hypoglycaemia and no body weight gain.

Supported by: Merck Sharp & Dohme Corp.

## 835

### China type 2 diabetes treatment status survey of treatment pattern of oral drugs users (China DiaSTAGE)

L. Ji<sup>1</sup>, J. Lu<sup>2</sup>, J. Weng<sup>3</sup>, W. Jia<sup>4</sup>, H. Tian<sup>5</sup>, D. Zhu<sup>6</sup>, X. Xing<sup>7</sup>, L. Guo<sup>8</sup>,  
<sup>1</sup>Peking University People's Hospital, <sup>2</sup>Chinese PLA General Hospital, Beijing, <sup>3</sup>The Third Affiliated Hospital Of Sun Yat-sen University, Guangzhou, <sup>4</sup>Shanghai 6th Hospital, Shanghai, <sup>5</sup>West China Hospital, Chengdu, <sup>6</sup>Nanjing Drum Tower Hospital, Jiangsu, <sup>7</sup>Peking Union Medical College Hospital, Beijing, <sup>8</sup>Beijing Hospital, Beijing, China.

**Background and aims:** Diabetes is a serious threat to public health. It is very important and cost effective to implement diabetes management guidelines strategically and systematically. This study provides valuable basic data of oral antidiabetic drug (OAD) therapy status for type 2 diabetes (T2D) patients in clinical practice which can contribute to future popularisation and implementation of Chinese T2D management guidelines.

**Materials and methods:** The China DiaSTAGE, a non-interventional, cross-sectional study, was conducted at 103 hospitals across the country. The study included outpatients with T2D who have received treatment for T2D for  $\geq 6$  months, have visited the investigated hospital for  $\geq 3$  months, have taken OADs (monotherapy or combined therapy) for  $\geq 3$  months; and who completed the study questionnaire. The first 5 patients who fulfilled the inclusion criteria were enrolled every day. Demography, medical history, current antidiabetic treatment regimen, treatment alteration within the last year, status of glycaemic control, frequency of hypoglycaemia within last month, presence of oedema and change in body weight were studied.

**Results:** The study included 9872 Chinese T2D patients of which 50.9% were males. Mean age was  $60.0 \pm 11.3$  years and BMI  $24.6 \pm 3.2 \text{ kg/m}^2$ . Medical history included hypertension (52.2%), dyslipidaemia (43.5%), coronary heart disease (16.7%), diabetic neuropathy (15.6%), diabetic retinopathy (13.9%), diabetic nephropathy (6.7%), stroke (6.0%) and diabetic foot (3.1%). Physician suggested diet control and exercise as a treatment regimen in 87.0% and 77.8% patients, respectively. Use of insulin secretagogues (70.2%), such as sulphonylureas (SU; 42.7%) or glinides (27.5%), was most common followed by metformin (53.7%), glucosidase inhibitors (35.9%), thiazolidinediones (17.2%) and DPP-IV inhibitors (0.8%). Dual-drug combination therapy was more common (45.4%) than monotherapy (35.8%) and combination therapy of  $\geq 3$  drugs (17.0%). Metformin/SU was the most common dual-drug com-

bination. Among monotherapies, 45.6% patients received insulin secretagogues (SU or glinides) and 30% received metformin. Within the past year, 31.9% of patients altered their treatment regimen, mostly once (70.6%), citing poor effectiveness as the main reason. Glycaemic targets of  $\text{FPG} \leq 7 \text{ mmol/L}$ ,  $\text{PPG} \leq 10 \text{ mmol/L}$ ,  $\text{HbA}_{1c} \leq 6.5\%$  and  $\text{HbA}_{1c} \leq 7\%$  were achieved in 51.4%, 53.5%, 27.8% and 39.6% patients, respectively. The last reported mean  $\text{HbA}_{1c}$  was  $7.22 \pm 1.49\%$ . Presence of hypoglycaemia within last month was reported by 14% patients. Overall, 3% patients reported severe hypoglycaemia, 5% reported oedema and 8.2% reported weight gain ( $2.8 \pm 2.6 \text{ kg}$ ).

**Conclusion:** In China, insulin secretagogues are the first choice of OADs which may be the reason for observed high incidence of hypoglycaemia and maybe limit glucose control. Poor effectiveness is reported as the main reason for the alteration of treatment regimen. These observations may serve as guidance for future diabetes management guidelines in China.

Supported by: Novartis Pharma

## 836

### Glucose control and oral hypoglycaemic agents in eight regions across China

T. Chen<sup>1</sup>, H. Tian<sup>1</sup>, L. Ji<sup>2</sup>, China DiaSTAGE group;

<sup>1</sup>Department of Endocrinology and Metabolism, West China Hospital of Sichuan University, Chengdu, <sup>2</sup>Department of Endocrinology and Metabolism, Peking University People's Hospital, Beijing, China.

**Background and aims:** Due to economic factors, physician's preference and so on, the regimens of OHA might be different in different region of China, which might affect blood glucose (BG) control. To better address these issues, the Chinese Diabetes Association conducted a survey on the OHA regimens and its association with BG control in different regions across China.

**Materials and methods:** In this multi-center, non-intervention, cross-sectional survey, 10 000 type 2 diabetes patients at more than 100 medical centers were investigated. Oral hypoglycemic regimens (OHA, e. g. the types of drugs, the combination of drugs), and the frequencies of hypoglycemia and severe hypoglycemia etc were evaluated. FBG less than  $7 \text{ mmol/L}$ , 2hPBG less than  $10 \text{ mmol/L}$  and  $\text{HbA}_{1c}$  less than 7% were defined as good BG control. The regions were divided into eight conventionally, that is, Northwest (Gansu), Northeast (Liaoning, Jilin, Heilongjiang), Northern coastal region (Beijing, Tianjin, Shandong), Middle reaches of the Yellow River (Shaanxi), Eastern coastal region (Shanghai, Jiangsu, Zhejiang), Middle reaches of the Yangtze River (Hunan, Hubei, Jiangxi), Southwest (Sichuan, Chongqing, Guizhou, Guangxi), and Southern coastal areas (Fujian, Guangdong and Hainan).

**Results:** 1. The levels of FBG, 2hPBG and  $\text{HbA}_{1c}$  of patients from Southern coast, Northern coast, East coast and Middle reaches of the Yangtze River, were lower than those from the Middle reaches of the Yellow River, Northeast and Northwest regions. The good control rates FBG, 2hPBG and  $\text{HbA}_{1c}$  were ranged from 49.9% to 56.5%, from 50.8 to 59.2%, and from 43.6 to 50.6% respectively. While in Northwest, Northeast and Middle Yellow River regions, the rates ranged from 26.8% to 36.0%, from 24.7% to 46.3%, from 20.1% to 35.8% respectively. Good control rates of FBG and 2hPBG in Southwest were about 50%, but that of  $\text{HbA}_{1c}$  was only 33.7%. 2. The incidence of hypoglycemia and severe hypoglycemia were significantly higher in Middle reaches of the Yellow River. 3. Further analysis indicated that in regions with bad BG control, the rates of diet and/or physical exercise were relatively lower, the rates of OHA combination therapy were relatively infrequent, the rates of sulphonylureas, glinides, glucosidase inhibitor medication were lower, and the rates of other drugs (traditional Chinese medicine, the mixture of Chinese and Western medicine, fixed formula compound preparation) were higher.

**Conclusion:** The overall status of blood glucose control in China was not optimistic, and which were even worse in regions of Northwest, Northeast and Middle reaches of the Yellow River. Relatively poor diet and physical exercise and the different choices of OHA regimen were potential contributing factors. Clinical practitioners should pay more attention to such issues in the future.



## PS 066 DPP-4 inhibitors: clinical studies

837

### Efficacy and safety of saxagliptin (SAXA) in patients with type 2 diabetes and a history of cardiovascular disease

W. Cook<sup>1</sup>, B. Bryzinski<sup>2</sup>, J. Slater<sup>3</sup>, M. Donovan<sup>3</sup>, E. Allen<sup>3</sup>;<sup>1</sup>Medical Affairs, AstraZeneca, Wilmington, DE, USA, <sup>2</sup>Research & Development, AstraZeneca, Wilmington, <sup>3</sup>Bristol-Myers Squibb, Princeton, Bristol-Myers Squibb, Princeton, USA

**Background and aims:** To determine the efficacy and safety of SAXA in patients (pts) with T2D and a history of CV disease, we conducted a subgroup analysis of pooled data from 5 phase 3 placebo-controlled 24-week studies: 2 studies of SAXA as monotherapy in drug-naïve pts and 1 study each of SAXA as add-on therapy to metformin, glyburide, or a thiazolidinedione.

**Materials and methods:** Pooled efficacy (glycated hemoglobin [HbA<sub>1c</sub>], fasting plasma glucose [FPG], 120-min postprandial glucose [PPG]) and safety data (adverse events [AEs], body weight) for SAXA 5 mg and placebo were analyzed in pts with a history of CV disease (previous event or diagnosis of CV disease) or no history of CV disease using analysis of covariance.

**Results:** In total, 882 pts received SAXA 5 mg and 799 received placebo. At week 24, improvements in HbA<sub>1c</sub>, FPG, and PPG were greater and more pts achieved a glycemic target (HbA<sub>1c</sub> <7%) with SAXA compared with placebo irrespective of CV disease history. There were no important treatment-by-subgroup interactions for SAXA. Differences in adjusted mean±SE reductions in HbA<sub>1c</sub> with SAXA were greater than placebo in pts with a history of CV disease (−0.64±0.14 [95% CI, −0.90 to −0.38]; Table). Similar results were seen in pts without CV disease (−0.68±0.05 [95% CI, −0.78 to −0.58]). Differences in adjusted mean change in body weight with SAXA vs placebo were +0.7 kg (CV disease history) and +0.5 kg (no CV disease history). The incidences of ≥1 AE with SAXA vs placebo were 70.3% vs 72.2%, respectively, in pts with CV disease history and 72.3% vs 70.2% in those without, and the incidences of ≥1 treatment-related AE were 18.9% vs 14.4% and 18.7% vs 17.0%, respectively. The rates of discontinuations for AEs for SAXA vs placebo were 2.7% vs 2.1% in those with and 3.4% vs 1.7% in those without CV disease history. Serious AEs were reported by 4.5% of SAXA-treated pts vs 7.2% of those receiving placebo in pts with and 3.1% vs 2.9% in pts without CV disease history. Two pts without CV disease history (n=1, SAXA 5 mg; n=1, placebo) experienced ≥1 treatment-related serious AE. There were 2 deaths in pts receiving placebo, 1 in each subgroup. The incidence of all reported hypoglycemic AEs was similar in pts with and without CV disease history (7.2% with SAXA vs 6.2% with placebo and 7.8% vs 6.7%, respectively). Confirmed hypoglycemia (fingerstick glucose ≤50 mg/dL and associated symptoms) was reported by 0 pts treated with SAXA and 2.1% of pts receiving placebo in subgroups with CV disease history and 0.5% vs 0.1%, respectively in those without CV disease history.

**Conclusion:** In this pooled analysis of phase 3 placebo-controlled trials, SAXA 5 mg was similarly effective with an adverse event rate similar to placebo in pts with T2D regardless of CV disease history. Glycemic efficacy at 24 weeks with SAXA 5 mg in patients with and without CV disease history

	CV Disease History		No CV Disease History	
	SAXA 5 mg	Mean±SE Difference vs Placebo (95% CI)*	SAXA 5 mg	Mean±SE Difference vs Placebo (95% CI)*
HbA <sub>1c</sub> , % <sup>†</sup>	n=110	n=96	n=746	n=680
Mean±SE adjusted change from baseline (95% CI)	−0.70±0.09 (−0.88 to −0.52)	−0.64±0.14 (−0.90 to −0.38)	−0.70±0.04 (−0.77 to −0.63)	−0.68±0.05 (−0.78 to −0.58)
FPG, mg/dL <sup>‡</sup>	n=110	n=96	n=756	n=686
Mean±SE adjusted change from baseline (95% CI)	−18.2±3.6 (−25.3 to −11.1)	−15.8±5.3 (−26.2 to −5.3)	−13.0±1.4 (−15.8 to −10.3)	−14.4±2.0 (−18.3 to −10.4)
PPG-120, mg/dL <sup>§</sup>	n=88	n=64	n=574	n=520
Mean±SE adjusted change from baseline (95% CI)	−54.5±6.9 (−68.1 to −40.9)	−38.2±10.7 (−59.1 to −17.3)	−52.0±2.8 (−57.5 to −46.6)	−41.4±3.9 (−49.2 to −33.7)
HbA <sub>1c</sub> <7%	n=110	n=96	n=747	n=680
Patients achieving target, %	43.6	21.8 (8.2 to 35.4)	35.1	15.7 (10.9 to 20.4)

\*n value in difference column reflects the number of patients in the placebo group.

<sup>†</sup>Drug-by-CV disease history interaction, *P*=0.9453, indicating no difference in treatment effect based on CV history.

<sup>‡</sup>Drug-by-CV disease history interaction, *P*=0.4137, indicating no difference in treatment effect based on CV history.

<sup>§</sup>Drug-by-CV disease history interaction, *P*=0.5164, indicating no difference in treatment effect based on CV history.

CV=cardiovascular; FPG=fasting plasma glucose; HbA<sub>1c</sub>=glycated hemoglobin; PPG-120=120-minute postprandial glucose; SAXA=saxagliptin.

Clinical Trial Registration Number: NCT00121641, NCT00316082, NCT00121667, NCT00295633, NCT00313313

Supported by: Bristol-Myers Squibb and AstraZeneca

838

### Efficacy and safety of saxagliptin (SAXA) in patients with type 2 diabetes stratified by cardiovascular risk factors

E. Allen<sup>1</sup>, J. Slater<sup>1</sup>, B. Bryzinski<sup>2</sup>, M. Donovan<sup>3</sup>, W. Cook<sup>4</sup>;<sup>1</sup>Medical Affairs, Bristol-Myers Squibb, Princeton, <sup>2</sup>Research & Development, AstraZeneca, Wilmington, <sup>3</sup>Biostatistics, Bristol-Myers Squibb, Princeton, <sup>4</sup>Medical Affairs, AstraZeneca, Wilmington, USA.

**Background and aims:** Patients (pts) with T2D have elevated risk of CV disease, are likely to have other comorbidities, and receive concomitant medications for those conditions. To determine if there is a difference in SAXA efficacy in T2D pts with vs without hypertension (HTN), statin use, or multiple CV risk factors, we conducted a subgroup analysis of pooled data from 5 phase 3 placebo (PBO)-controlled 24-week studies: 2 studies of SAXA as monotherapy in drug-naïve pts and 1 study each of SAXA as add-on therapy to metformin, glyburide, or a thiazolidinedione.

**Materials and methods:** Pooled efficacy (glycated hemoglobin [HbA<sub>1c</sub>], fasting plasma glucose [FPG], 120-min postprandial glucose [PPG]) and safety data (adverse events [AEs], serious AEs, hypoglycemia, body weight) for SAXA 5 mg and PBO were analyzed in subgroups defined by the presence of HTN/no HTN, statin use/no statin use, and multiple (≥2 or 0-1) CV risk factors (HTN, dyslipidemia, smoking, family history of CV disease) using analysis of covariance.

**Results:** In total, 882 pts received SAXA 5 mg and 799 received PBO. At week 24, improvements in glycemic control were greater with SAXA vs PBO in all subgroups (Table). There were no important treatment-by-subgroup interactions for SAXA (Table). In all subgroups, glycemic target of HbA<sub>1c</sub> <7% was achieved by more pts treated with SAXA vs PBO (difference in proportions vs PBO [95% CI], using Mantel-Haenszel rate difference estimate: HTN, 16.7% [10.6%-22.7%]; no HTN, 16.3% [9.6%-23.0%]; statin use, 18.2% [9.0%-27.3%]; no statin use, 15.8% [10.6%-20.9%]; ≥2 CV risk factors, 16.7% [10.4%-23.0%]; 0-1 CV risk factor, 16.2% [9.8%-22.6%]). Overall incidence of ≥1 AE with SAXA vs PBO was as follows in each subgroup: 71.0% vs 72.5% (HTN), 73.7% vs 67.9% (no HTN), 77.1% vs 75.5% (statin use), 70.7% vs 68.8% (no statin use), 75.4% vs 73.0% (≥2 CV risk factors), and 68.6% vs 67.9% (0-1 CV risk factor). Incidence of ≥1 serious AE was similar for SAXA vs PBO in subgroups with HTN (3.6% vs 4.5%), no HTN (3.1% vs 2.0%), with statin use (4.7% vs 3.8%), no statin use (3.0% vs 3.2%), with ≥2 CV risk factors

(3.8% vs 4.1%), and with 0–1 CV risk factor (2.9% vs 2.6%). There were no deaths in the SAXA groups and 2 deaths in the PBO group (HTN subgroup [n=2]; no statin use subgroup [n=2];  $\geq 2$  CV risk factors subgroup [n=2]). Incidences of all reported hypoglycemia ranged from 6.7%–11.2% with SAXA vs 6.3%–7.2% with PBO. Symptomatic confirmed hypoglycemia (glucose  $\leq 50$  mg/dL) was  $<1\%$  in all subgroups. Differences in adjusted mean change in body weight with SAXA vs PBO ranged from 0.4–0.7 kg.

**Conclusion:** SAXA 5 mg was effective with an AE rate profile similar to PBO in pts with T2D irrespective of concomitant HTN, statin use, or number of CV risk factors. Concomitant statin use was not associated with a lower HbA<sub>1c</sub> reduction. Glycemic efficacy at 24 weeks with SAXA 5 mg in 5 pooled studies.

	Hyper-tension	No Hyper-tension	Statin Use	No Statin Use	$\geq 2$ CV Risk Factors	0 or 1 CV Risk Factor
HbA <sub>1c</sub> %	n=457	n=402	n=211	n=650	n=459	n=402
Mean difference in adjusted change from baseline vs PBO (95% CI)	-0.69 (-0.82 to -0.57)	-0.66 (-0.80 to -0.52)	-0.70 (-0.89 to -0.52)	-0.66 (-0.77 to -0.56)	-0.73 (-0.85 to -0.60)	-0.62 (-0.75 to -0.48)
P value for drug-by-subgroup interaction	0.7962		0.9242		0.4922	
FPG, mg/dL	n=461	n=408	n=211	n=660	n=463	n=408
Mean difference in adjusted change from baseline vs PBO (95% CI)	-14.2 (-19.2 to -9.2)	-15.8 (-21.2 to -10.3)	-16.0 (-23.3 to -8.8)	-14.3 (-18.5 to -10.0)	-14.7 (-19.8 to -9.7)	-14.7 (-20.1 to -9.4)
P value for drug-by-subgroup interaction	0.7352		0.8592		0.6504	
PPG-120 min, mg/dL	n=351	n=313	n=158	n=508	n=354	n=312
Mean difference in adjusted change from baseline vs PBO (95% CI)	-40.7 (-50.5 to -30.9)	-41.9 (-52.6 to -31.2)	-45.7 (-60.2 to -31.2)	-39.9 (-48.2 to -31.6)	-36.1 (-46.0 to -26.2)	-47.0 (-57.5 to -36.5)
P value for drug-by-subgroup interaction	0.3938		0.5438		0.0611	

CV=cardiovascular; FPG=fasting plasma glucose; HbA<sub>1c</sub>=glycated hemoglobin; PBO=placebo; PPG-120 min=120-minute postprandial glucose; SAXA=saxagliptin.

Clinical Trial Registration Number: NCT00121641, NCT00316082, NCT00121667, NCT00295633, NCT00313313  
Supported by: Bristol-Myers Squibb and AstraZeneca

## 839

### An integrated, multi study analysis of alogliptin safety

M. Hisada, M. Munsaka, J. Streit, N. Smith;  
Takeda Global Research & Development Center, Inc., Deerfield, USA.

**Background and aims:** Alogliptin (ALO) is a selective inhibitor of dipeptidyl peptidase-4 for the treatment of type 2 diabetes mellitus. We performed an integrated analysis of controlled phase 2 and 3 studies to evaluate the safety of ALO.

**Materials and methods:** Subjects who received at least one dose of study drug were included in the analysis of 11 pooled studies and were categorized by treatment assignment as ALO (n=4162) or comparator (including placebo) (Com; n=1855).

**Results:** Demographics were similar between groups. The majority of subjects were aged  $<65$  years (ALO, 78%; Com, 72%), female (both 51%), and white (ALO, 73%; Com, 71%), with BMI  $\geq 30$  kg/m<sup>2</sup> (ALO, 55%; Com, 53%), and had mild renal impairment (MDRD: eGFR  $\geq 60$  -  $< 90$  mL/min/1.73 m<sup>2</sup> criteria) (both 68%); mean baseline A1c was 8.3% (both). A lower proportion of ALO vs Com subjects required hyperglycemic rescue (11% vs 21%). Similar proportions of subjects in both groups experienced  $\geq 1$  adverse event (AE) (ALO, 65%; Com, 64%). The only AEs occurring at  $\geq 5\%$  in either group were urinary tract infection (ALO, 4.9%; Com 5.2%) and headache (both 5.0%). Serious AEs were reported by 4% of subjects in each group and deaths attributed to AEs occurred at 0.1% for both groups. A lower proportion of ALO vs Com subjects experienced a major adverse cardiovascular event (MACE: 0.3% ALO vs 0.5% Com); the hazard ratio for MACE for ALO vs Com was 0.65 (upper bound of 1-sided 97.5% CI = 1.41). Incidence of hepatic enzyme elevation (ALO, 0.4%; Com, 0.3%) was low in both groups. Rare events of rash and pruritus were reported with ALO. Four subjects in the ALO group (0.1%) reported acute pancreatitis as compared to 0 in the Com group.

**Conclusion:** This analysis suggests that ALO is safe and well tolerated with a safety profile similar to that of other DPP-4 inhibitors.

Supported by: Takeda Global Research & Development Center, Inc.

## 840

### Similar glucose control over 52 weeks with alogliptin vs glipizide in older, mildly hyperglycaemic patients with type 2 diabetes

P. Fleck<sup>1</sup>, C. Wilson<sup>1</sup>, J. Rosenstock<sup>2</sup>;

<sup>1</sup>Takeda Global Research & Development Center, Inc., Deerfield, <sup>2</sup>Dallas Diabetes and Endocrine Center, Dallas, USA.

**Background and aims:** Most studies on the effects of DPP-4 inhibitors in older people with type 2 diabetes mellitus (T2DM) have been post-hoc analyses. This study prospectively evaluated the efficacy and safety of alogliptin (ALO) vs glipizide (GLIP) in a well-defined older population with T2DM over 1 year of treatment.

**Materials and methods:** Eligible subjects with T2DM had to be aged 6590 years who had failed diet/exercise therapy alone (A1c 6.5% - 9.0%) or were inadequately controlled despite oral antidiabetic monotherapy (A1c 6.5% - 8.0%). The study comprised a screening period (2 wks), treatment period (52 wks), and visits at end-of-study (or early termination) and follow-up (2 wks later). Those on oral antidiabetic monotherapy underwent a washout period (4 wks) prior to randomization. Subjects (441 total) were randomized to once-daily ALO 25 mg (n=222) or GLIP 5 mg titrated to 10 mg, if needed (n=219). Hypoglycemic episodes were identified via stringent predefined criteria and procedures.

**Results:** Baseline characteristics were similar between the groups (mean A1c, 7.5%; diabetes duration, 6.1 years; BMI, 29.8; weight, 78.7 kg). In the primary analysis, A1c least squares (LS) mean changes from baseline to week 52 were 0.14% with ALO and 0.09% with GLIP, demonstrating noninferiority of ALO relative to GLIP (LS mean difference = -0.05%; 1-sided 97.5% confidence interval [CI]: infinity, 0.13%). More substantial A1c reductions were observed among subjects who completed the study: 0.42% with ALO and -0.33% with GLIP, with noninferiority again confirmed (LS mean difference = 0.09%; 1-sided 97.5% CI: infinity, 0.07%). A1c decreases were greater among subjects with baseline A1c  $\geq 8.0\%$  (0.32% with ALO and 0.34% with GLIP) vs those whose baseline A1c was  $<8.0\%$  (0.07 and -0.04%, respectively). Overall, alogliptin was safe and well tolerated. Hypoglycemic episodes were notably less frequent with ALO (5.4% of subjects reporting 31 episodes) vs GLIP (26.0% of subjects reporting 232 episodes). Most episodes were mild or moderate (BG  $<70$  mg/dL); severe hypoglycemia (BG  $<70$  mg/dL, requiring assistance) occurred in 3 subjects (all GLIP). ALO also resulted in significant weight decrease vs GLIP (0.62 vs 0.60 kg, respectively, at week 52;  $P<0.001$ ).

**Conclusion:** Alogliptin maintains comparable glucose control to glipizide but with substantially lower risk of hypoglycemia and without weight gain over 1 year of treatment in older patients with T2DM.

Clinical Trial Registration Number: NCT00707993

Supported by: Takeda Global Research & Development Center, Inc.

## 841

### Alogliptin plus metformin combination therapy vs alogliptin or metformin monotherapy for type 2 diabetes mellitus

R. Pratley<sup>1</sup>, C. Wilson<sup>2</sup>, P. Fleck<sup>2</sup>;

<sup>1</sup>Florida Hospital Diabetes and Translational Research Institute and Sanford|Burnham Institute, Orlando, <sup>2</sup>Takeda Global Research & Development Center, Inc., Deerfield, USA.

**Background and aims:** The efficacy and safety of alogliptin (ALO) plus metformin combination therapy (ALO+MET) in doses of 12.5/500 and 12.5/1000 mg BID vs monotherapy with ALO 12.5 mg BID (ALO12.5) or MET 500 or 1000 mg BID (MET500 or MET1000) was evaluated in a 7-arm study of 784 subjects with type 2 diabetes mellitus inadequately controlled with diet and exercise alone; 2 arms, placebo and ALO 25 mg QD, were included for the purposes of secondary analyses.

**Materials and methods:** In the primary efficacy analysis, the 2 combination therapy regimens were compared with their monotherapy regimens (ALO+MET 12.5/500 vs ALO12.5 and MET500, and ALO+MET 12.5/1000 vs ALO12.5 and MET1000).

**Results:** The majority of subjects were white (72%), women (52%), mean age of 54 years, body mass index of 31, diabetes duration of 4 years, and baseline A1c of 8.4%. Reductions in A1c at week 26 (primary endpoint) were 1.22% and 1.55% with ALO+MET 12.5/500 and 12.5/1000, vs 0.56%, 0.65%, and 1.11% with ALO12.5, MET500, and MET1000 ( $P<0.001$  for all comparisons). Significantly more subjects achieved A1c  $<7\%$  with ALO+MET (47.1% and 59.5% with 12.5/500 and 12.5/1000) vs monotherapy with ALO12.5, MET500, or MET1000 (20.2%, 27.2%, and 34.3%) ( $P<0.01$  for all comparisons). Reductions in FPG were -31.7 and 45.9 mg/dL with ALO+MET

12.5/500 and 12.5/1000, vs 9.7, 11.5, and 31.9 mg/dL with ALO12.5, MET500, and MET1000 ( $P<0.05$  for all comparisons). Significantly fewer ALO+MET subjects were rescued for hyperglycemia: 12.3% and 2.6% with 12.5/500 and 12.5/1000, vs 17.3%, 22.9%, 10.8% with ALO12.5, MET500, and MET1000 ( $P<0.05$  for all comparisons). Greater reductions in proinsulin/insulin ratios and increases in HOMA-BCF (beta cell function) with ALO+MET vs ALO or MET monotherapy were observed. Modest weight decreases were observed with ALO+MET and MET, while ALO monotherapy was weight neutral. ALO and MET alone demonstrated the expected safety profiles, and the combination doses were well tolerated, having safety profiles similar to that of the monotherapy regimens. Overall, few subjects reported hypoglycemic events.

**Conclusion:** In summary, ALO+MET combination therapy provided significantly better glycemic control than ALO or MET monotherapy, with a safety profile consistent with its individual components.

*Clinical Trial Registration Number:* NCT01023581

*Supported by:* Takeda Global Research & Development Center, Inc.

## 842

### Effect of alogliptin in combination with pioglitazone on glycaemic control by baseline HbA<sub>1c</sub>

C. Wilson, P. Fleck;

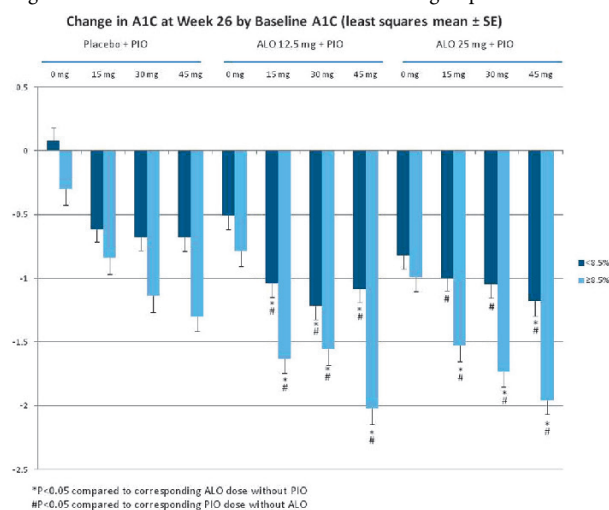
Takeda Global Research & Development Center, Inc., Deerfield, USA.

**Background and aims:** This phase 3, randomised, double-blind, placebo-controlled, 12-treatment arm study assessed the efficacy and safety of alogliptin (ALO) alone or combined with pioglitazone (PIO) in patients with type 2 diabetes on metformin with inadequate glycemic control.

**Materials and methods:** Treatment arms included ALO alone at doses of 12.5 or 25 mg QD; PIO alone at doses of 15, 30, or 45 mg QD; combinations of each ALO dose with each PIO dose; and a placebo arm. The primary analysis compared PIO alone (doses pooled; 387) with ALO 12.5 mg + any dose of PIO ( $n=390$ ) or ALO 25 mg + any dose of PIO ( $n=390$ ). The change from baseline A1C at week 26 by baseline A1C was determined using 2 subgroups:  $<8.5\%$  and  $\geq 8.5\%$ .

**Results:** Subjects in the combination groups achieved significantly larger decreases from baseline in A1C levels at week 26 compared with subjects in the PIO alone group ( $P<0.001$ ); greater reductions were observed for subjects with higher baseline A1C levels. Similarly, the majority of the ALO + PIO arms exhibited a statistically significant decrease in A1C levels at week 26 compared with corresponding doses of ALO alone and PIO alone ( $P<0.05$ ), regardless of baseline A1C; however, decreases were larger in subjects with higher baseline A1C levels (Figure). The incidence of adverse events (AEs) was similar across all 12 arms, ranging from 54.3% (placebo) to 69.2% (ALO 25 mg + PIO 15 mg); serious AEs ranged from 0.8% (PIO 15 mg and ALO 25 mg + PIO 15 mg) to 7.8% (PIO 45 mg). Few subjects discontinued due to an AE, with the most occurring in the PIO 45 mg arm (7/129; 5.4%). Overall, few subjects reported hypoglycemic events.

**Conclusion:** In conclusion, ALO + PIO was significantly more effective than either monotherapy, with greater reductions being achieved in patients with higher baseline A1C levels in all active treatment groups.



*Clinical Trial Registration Number:* NCT00328627

*Supported by:* Takeda Global Research & Development Center, Inc.

## 843

### Differential effects of vildagliptin and glimepiride on the glucose fluctuation in patients with type 2 diabetes mellitus assessed with continuous glucose monitoring

Y.-L. He<sup>1</sup>, G. Foteinos<sup>1</sup>, S. Neelakantham<sup>2</sup>, D. Mattapalli<sup>2</sup>, K. Kulmatycki<sup>1</sup>, T. Forst<sup>3</sup>, A. Taylor<sup>1</sup>;

<sup>1</sup>Translational Science, Novartis, Cambridge, USA, <sup>2</sup>Novartis, Hyderabad, India, <sup>3</sup>Institute for Clinical Research and Development, Mainz, Germany.

**Background and aims:** Vildagliptin is a potent, selective and orally active inhibitor of dipeptidylpeptidase-IV (DPP-4), which is approved for the treatment of type 2 diabetes mellitus (T2DM). Vildagliptin has demonstrated a low risk of hypoglycaemia, a major safety concern with glucose lowering therapy. Although it is well known that hyperglycaemia is closely related to the development of macro- and microvascular complications of diabetes, an increasing number of studies have shown that glucose fluctuation or variability is more important than chronic sustained hyperglycaemia in generating oxidative stress and contributing to the diabetes complications. The objective of this study was to assess whether or not there is a difference in the effects of vildagliptin and glimepiride on glucose fluctuation in patients with T2DM using continuous glucose monitoring (CGM).

**Materials and methods:** This was an open-label, randomised, crossover study in patients with T2DM. A total of 24 patients (Age:  $56.6 \pm 5.76$  yrs, baseline HbA<sub>1c</sub>:  $7.55 \pm 0.50\%$ ) were enrolled and all completed the study. Each patient received two 5-day treatments (vildagliptin 50 mg bid or glimepiride 2 mg qd) in a cross-over manner with ~14 day washout period. Blood glucose concentrations were measured using a CGM device during baseline and 5 day treatment, and glucose fluctuation parameters [standard deviation (SD) of the rate of change, interquartile range (IQR), rate of change in the median curve (RCMC), mean amplitude of glucose excursion (MAGE)] were estimated at baseline and day 5. The relationship between the ratio of urinary excretion rate of 8-iso-prostaglandin F<sub>2a</sub> and creatinine and MAGE was also explored.

**Results:** Both vildagliptin and glimepiride reduced postprandial glucose levels by ~15%, based on both CGM data and plasma glucose concentrations, which is consistent with the magnitude of effects for both agents reported in previous studies. When comparing the effects of vildagliptin and glimepiride on the glucose fluctuation, vildagliptin showed 26% lower RCMC ( $P = 0.0082$ ), 19% lower MAGE ( $P = 0.1076$ ) and 23% lower IQR ( $P = 0.0462$ ) than glimepiride. It is interesting to note that vildagliptin led to statistically significant lower RCMC and IQR, however, the reduction in MAGE did not reach statistical significance. MAGE is a parameter derived from the glucose profiles and the variability of MAGE parameter tends to be higher due to the derivative nature. The inability to demonstrate a statistically significant difference could be attributed to the small sample size. There was no apparent association between the ratio of 8isoprostaglandin F<sub>2a</sub> and creatinine and MAGE detected. Both vildagliptin and glimepiride were generally safe and well tolerated. Only one subject receiving treatment with glimepiride had a symptomatic hypoglycaemia event which required treatment.

**Conclusion:** Vildagliptin treatment is associated with significant lower glucose fluctuation than Glimepiride in patients with T2DM. These results suggest that vildagliptin may have the potential to offer long term beneficial effects for patients with T2DM in preventing the development of macro- or microvascular complications of diabetes.

*Clinical Trial Registration Number:* NCT01262586

*Supported by:* Novartis Pharma

## 844

### Comparison of glycaemic variability in patients with type 2 diabetes given sitagliptin or voglibose: a continuous glucose monitoring (CGM)-based pilot study

C. Seo, M. Sakamoto, R. Nishimura, D. Tsujino, K. Andou, A. Morimoto, K. Utsunomiya;

Diabetes, Metabolism and Endocrinology, Jikei University School of Medicine, Minato-ku, Nishishinbashi, Tokyo, Japan.

**Background and aims:** To compare glycemic variability in patients with type 2 diabetes given sitagliptin (S) or voglibose (V).

**Methods:** The study included a total of 15 type 2 diabetic patients aged 20 years old or older but younger than 80 years of age. These patients were randomly allocated to S 50 mg/day or V 0.9 mg/day as monotherapy or as addition to a sulfonylurea, thiazolidinedione or biguanide and were hospitalized



for evaluation by CGM for a 4-day period after 2 months of treatment with either regimen. At hospital discharge, the patients were crossed over to the other regimen, and were hospitalized again after 2 months of treatment for evaluation by CGM for a 4-day period. They were given the same meals on days 2 and 3 during both hospitalizations and were not allowed to take any other additional oral hypoglycemic agent during the study. Variables compared by using the CGM data available from day 2 of either hospitalization with the Wilcoxon signed-rank test included: 1) 24-hour mean glucose levels; 2) standard deviations (SD) of 24-hour; 3) mean amplitude of glycemic excursions (MAGE); 4) preprandial glucose levels; 5) postprandial peak glucose levels; 6) range of glucose increase from preprandial to postprandial peak glucose levels; 7) time from preprandial to postprandial peak glucose levels; and 8) postprandial glucose increase gradients. All statistical analyses were performed by using SPSS ver. 19. The present study was conducted with the approval of the Ethics Committee of Jikei University School of Medicine.

**Results:** The median age of the patients was 59 years (25–75 percentiles, 43–70), their median HbA<sub>1c</sub> (international standards value) 7.1% (6.8–8.1), and their median BMI 26.6 kg/m<sup>2</sup> (25.2–30.3). Their 24-hour mean glucose levels with S and V were significantly ( $P = 0.020$ ) different at 128 and 143 mg/dL, respectively, with their glucose levels being 119 and 130 before breakfast ( $P = 0.035$ ), 108 and 116 before lunch, and 105 and 112 mg/dL before dinner, respectively. The time from preprandial to postprandial peak glucose levels was significantly longer after dinner with V than with S, with that after breakfast, lunch and dinner being 65 and 90, 100 and 140, and 90 and 120 minutes ( $P = 0.013$ ), respectively. The postprandial glucose increase gradient was significantly lower with V after breakfast and dinner, with that after breakfast, lunch and dinner being 1.07 and 0.84 ( $P = 0.047$ ), 0.53 and 0.35, 1.00 and 0.68 ( $P = 0.036$ ) mg/dL/min, respectively. However, no difference was seen between S and V with regard to 24hSD (24.9 and 27.1), MAGE (72.3 and 65.3), postprandial peak glucose levels (morning, 193 and 208; lunch, 157 and 178; and dinner, 188 and 197 mg/dL), and range of postprandial glucose increase (after breakfast, 91 and 80; after lunch, 54 and 42; and after dinner, 87 and 90 mg/dL).

**Conclusion:** A CGM-based study of glycemic variability with S and V revealed that S significantly lowered 24-hour mean glucose levels and glucose levels before breakfast compared to V, while the time from preprandial to postprandial peak glucose levels was significantly longer, and the glucose increase gradient significantly lower after breakfast and dinner, with V compared to S.

Clinical Trial Registration Number: UMIN000004916

Supported by: Japan Diabetes Foundation

## 845

**Real life evaluation of DPP4-inhibitors versus other oral antidiabetics in elderly patients with type 2 diabetes mellitus treated with metformin: results from the HYPOCRAS study**

A. Penforis<sup>1</sup>, I. Bourdel-Marchasson<sup>2</sup>, S. Quéré<sup>3</sup>, S. Dejager<sup>3</sup>;

<sup>1</sup>University of Franche-Comté, CHU of Besançon, <sup>2</sup>University Bordeaux, CHU of Bordeaux, <sup>3</sup>Biostatistics & Clinical Research departments, Novartis Pharma SAS, Rueil Malmaison, France.

**Background and aims:** The risk of hypoglycaemia is likely the major complicating factor of antidiabetic treatment in elderly patients and diabetes management is more challenging, although it has received little attention resulting in a paucity of data regarding the use of pharmacological agents in this age group. In this prospective observational study, we evaluated the real life use of DPP4-i vs other oral anti-diabetic drugs (OAD) in elderly patients with T2DM which was not optimally controlled on metformin alone.

**Materials and methods:** Between Sep 2009 and March 2011, 1317 patients  $\geq 65$  years and with HbA<sub>1c</sub>  $\geq 6.5\%$  on metformin were recruited by 663 general practitioners (GP) in France. 2 cohorts were constituted on the basis of the GP decision at the 1<sup>st</sup> visit: to add a DPP4-i or an OAD, and criteria of choice were collected. All patients were followed at 3 (M3) and 6 months (M6) to assess glycaemic control and the rate of reported hypoglycaemic events (HEs). 1188 completed the 6 months follow-up.

**Results:** 78% of patients received a DPP4-i ( $n = 931$ ) vs 22% an OAD ( $n = 257$ ); among these, 63.5% received a SU/glinide, 30% a TZD and 6.6% an  $\alpha$ -glucosidase inhibitor. Demographic characteristics were comparable at baseline (BL) between the 2 cohorts, with 61% of men, mean age of 71 yrs, 7 yrs of disease duration, 5.5 yrs of metformin use and mean BL HbA<sub>1c</sub> of 7.9%. 39% of patients were obese, 13% were smokers, 58% had hypertension, 58% dyslipidemia and about 44% of patients had at least one micro- or macrovascular complication. Despite the lack of difference in the populations

included in the 2 cohorts, GP declared significant differences in the criteria of choice for the second OAD, suggesting that this choice is essentially based on GP's beliefs and experience rather than on actual patients' characteristics. The proportion of patients reporting  $\geq 1$  HE over 6 months was significantly higher in the OOAD cohort: 20.1% vs 6.4% of patients in the DPP4i cohort ( $P < 0.001$ ). While severe HEs were rare in both cohorts, there were significantly more frequent with OOAD (2.4%) vs DPP4-i (0.1%;  $P = 0.001$ ). Further, among patients experiencing  $\geq 1$  HE, patients in the OOAD cohort were more likely to report multiple events with 16% reporting  $\geq 4$  HEs vs 5.5% of the DPP4i patients. Glucose control improved similarly in both cohorts, with HbA<sub>1c</sub> decreasing from a mean BL of 7.9% to 7.1% at M3 (in both cohorts) and to 7.0% (OOAD) and 6.9% (DPP4i) at M6 ( $P = 0.03$ ). However "Success of bitherapy" predefined as reaching the 7% target without any HE at M6, was significantly higher in the DPP4i cohort (59.7%) vs the OOAD cohort (45.5%;  $P < 0.001$ ). Over the course of 6 months, more patients discontinued from the OOAD treatment (6.6%) vs DPP4i (1.6%;  $P < 0.001$ ).

**Conclusion:** The population included in this large cohort study has similar characteristics than the representative sample of the French elderly population described in ENTRED study. While adding a DPP4i or OOAD to elderly patients with T2DM poorly controlled on metformin leads to a similar HbA<sub>1c</sub> decrease at M6, the percentage of patients reaching 7% target without HE is significantly greater with DPP4i. In ENTRED, 5% of patients taking SU or glinides reported  $\geq 1$  severe HE over a 1-year period, which is well consistent with the 2.4% rate seen in HYPOCRAS over 6 months.

Supported by: Novartis Pharma

## 846

**Contributing factors for initiation of GLP-1 agonist, DPP-4 inhibitor or insulin after metformin or sulfonylurea in US**

S. Schwartz<sup>1</sup>, M. Fournier<sup>2</sup>, H. Wang<sup>3</sup>;

<sup>1</sup>R&D, Main line Health System, Wynnewood, USA, <sup>2</sup>R&D, Sanofi, Paris, France, <sup>3</sup>R&D, Sanofi, Bridgewater, USA.

**Background and aims:** Therapeutic options for Type 2 diabetes after metformin (MET) or sulfonylurea (SU) include GLP-1 agonist (GLP-1), DPP-4 inhibitor (DPP-4) or Insulin. Contributing factors to the choice of therapy are not well understood.

**Materials and methods:** Patient cohorts stable on MET or SU ( $\geq 6$  months) in 2008 and 2009, as baseline (BL), were identified from the IMPACT<sup>TM</sup> claims database and evaluated for the initiation of GLP-1, DPP-4 or insulin in the subsequent year of 2009 and 2010, respectively.

**Results:** Stable MET or SU users were identified in 2008 ( $n=153,582$ ) and 2009 ( $n=148,704$ ). Rate of GLP-1 use was 1.3% to 1.8% and DPP-4 use was 5.4% to 6.1% from 2009 to 2010. Yearly insulin initiation rate remained stable at 3.5%. Mean A1c at BL was 8.1% in those who initiated insulin in 2009 and 8.4% in 2010. Mean A1c at BL was 7.5% for GLP-1 and 7.6% for DPP-4 initiators. At BL, insulin initiators were 85% on MET, 75% on SU and 3 out of 4 on  $\geq 2$  oral agents while GLP-1 and DPP-4 were initiated with 90% BL MET use, less with SU (60%) and two thirds on  $\geq 2$  oral agents. Insulin and DPP-4 initiators had a similar mean age of 57 years, while GLP-1 initiators were about 5 years younger, with more female (51% versus 40% and 43% for DPP-4 and Insulin, respectively) and nearly twice as many medical claims for obesity (21% vs 11% and 13%, respectively). In comparison with DPP-4, insulin initiators had more BL comorbidities, e.g. twice as many renal disease (8% for insulin vs 4% for DPP-4), 30% higher cardiovascular disorders (20% vs 15%) and over 70% more hospital admissions (16% vs 9%). GLP-1 initiators had the least BL comorbidity burden with 3% renal disease, 10% cardiovascular disorder and 8% hospital admissions.

**Conclusion:** Compared to GLP-1 or DPP-4 therapies, insulin is initiated in patients with a high A1c value. GLP-1 is more likely to be initiated in patients with younger age, female gender, lower comorbidity and more obesity.

Supported by: Sanofi

## PS 067 DPP-4 inhibitors I

### 847

#### Linagliptin added to sulphonylurea or $\alpha$ -glucosidase inhibitor therapy provides similar long-term safety and efficacy to metformin in Japanese patients with type 2 diabetes

N. Inagaki<sup>1</sup>, H. Watada<sup>2</sup>, M. Murai<sup>3</sup>, T. Kagimura<sup>3</sup>, A. Emser<sup>4</sup>, Y. Gong<sup>4</sup>, S. Patel<sup>5</sup>, H.-J. Woerle<sup>4</sup>;

<sup>1</sup>Department of Diabetes and Clinical Nutrition, Kyoto University Graduate School of Medicine, Kyoto, Japan, <sup>2</sup>Department of Metabolism and Endocrinology, Juntendo University Graduate School of Medicine, Tokyo, Japan, <sup>3</sup>Boehringer Ingelheim, Tokyo, Japan, <sup>4</sup>Boehringer Ingelheim, Ingelheim, Germany, <sup>5</sup>Boehringer Ingelheim, Bracknell, UK.

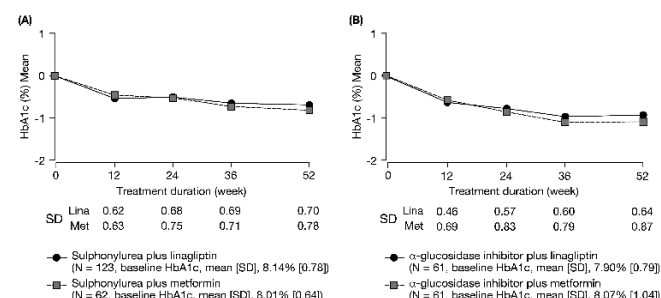
**Background and aims:** To evaluate the long-term safety and efficacy of oral linagliptin 5 mg once daily (Lina) vs metformin (Met), in patients with type 2 diabetes (T2DM) and insufficient glycaemic control despite background monotherapy with an approved oral antidiabetic drug (OAD).

**Materials and methods:** In total, 574 patients with HbA1c  $\geq 7\%$  to  $\leq 10\%$  entered this 52-week, open-label, multicenter, parallel-group trial. After a 2-week placebo run-in, patients on sulphonylurea (SU) or  $\alpha$ -glucosidase inhibitor (A-GI) were randomised to receive either Lina (once daily, 5mg) or Met (twice- or three-times daily, up to 2250 mg/day) as add-on therapy. Additionally, patients on biguanide, glinides, or glitazone received Lina add-on therapy.

**Results:** At week 52, add-on therapy with Lina and Met significantly reduced mean HbA1c levels, from a baseline of 7.9% through 8.1%, in all groups. Add-on therapy with Lina was as effective as Met after 52 weeks: adjusted mean change from baseline HbA1c was  $-0.66\%$  (95% confidence interval, CI,  $-0.81$ ,  $-0.51$ ) for Lina + SU ( $n=123$ ) vs  $-0.84\%$  (CI,  $-1.02$  to  $-0.65$ ) for Met + SU ( $n=62$ ), resulting in a non-significant treatment difference of  $0.18\%$  (CI,  $-0.03$  to  $0.39$ ); corresponding results were  $-0.92\%$  (CI,  $-1.10$  to  $-0.73$ ) for Lina + A-GI ( $n=61$ ) vs  $-1.00\%$  (CI,  $-1.17$  to  $-0.83$ ) for Met + A-GI ( $n=61$ ), resulting in a non-significant treatment difference of  $0.09\%$  (CI,  $-0.13$  to  $0.30$ ). Descriptive statistics demonstrate the consistent and sustained HbA1c decline over the 52-week treatment period (Figure). Adverse events (AE) were mostly mild or moderate and the rates were comparable across all groups: in SU-treated patients, AEs were reported in 82% of the Lina group and 70% of Met group, whilst in A-GI-treated patients, rates were 80% and 85%, respectively. The occurrence of hypoglycaemic events was similar for Lina and Met, and all were mild in intensity, except one event in the SU + Met group (moderate). In the A-GI group, hypoglycaemic events were reported in 2% (1/61) in the Lina add-on group, and 3% (2/61) receiving Met add-on. For those on SU background, corresponding rates were increased to 14% (17/124) of patients receiving add-on Lina vs 16% (10/63) receiving add-on Met.

**Conclusion:** Once-daily linagliptin provides well-tolerated, effective add-on therapy leading to significant HbA1c reductions, similar to metformin, over 52 weeks in Japanese patients with inadequately controlled T2DM despite monotherapy with an approved OAD.

Change from baseline HbA1c over time in patients receiving background therapy with sulphonylurea (A) or  $\alpha$ -glucosidase inhibitor (B) plus add-on linagliptin or metformin



Clinical Trial Registration Number: NCT01204294

Supported by: Boehringer Ingelheim

### 848

#### Safety and efficacy of linagliptin plus basal insulin combination therapy in a vulnerable population of elderly patients (age $\geq 70$ years) with type 2 diabetes

H.-J. Woerle<sup>1</sup>, D. Neubacher<sup>2</sup>, S. Patel<sup>3</sup>, M. von Eynatten<sup>1</sup>;

<sup>1</sup>Boehringer Ingelheim, Ingelheim, Germany, <sup>2</sup>Boehringer Ingelheim, Biberach, Germany, <sup>3</sup>Boehringer Ingelheim, Bracknell, UK.

**Background and aims:** Elderly patients with type 2 diabetes (T2D) are commonly characterised by longer disease duration and diminished  $\beta$ -cell capacity, which often requires combination therapy with basal insulin. Increasing the insulin dose is one option to improve glycaemic control, but this often presents a higher risk of hypoglycaemia, which increases overall safety concerns in this vulnerable population. Elderly patients may therefore benefit from an alternative option, such as an additional oral antidiabetic drug that can be administered safely in combination with their background basal insulin. Linagliptin is a recently approved oral DPP-4 inhibitor that has shown clinically meaningful efficacy and an overall safety profile similar to placebo in commonly used oral antidiabetic treatment regimens. It also has a once-daily, single-dose strength posology without the need for dose adjustment in patients with hepatic and/or renal impairment, which allows for convenient dosing and may support patient adherence.

**Materials and methods:** Two Phase 3 studies evaluating linagliptin vs. placebo as add-on therapy to basal insulin and as T2D management in elderly patients were eligible for a pre-specified pooled analysis exploring its safety and efficacy in combination with basal insulin in elderly patients (age  $\geq 70$  years) with T2D. The primary efficacy endpoint was change from baseline to week 24 in HbA1c. Safety and tolerability based on adverse events (AEs) were also assessed.

**Results:** A total of 247 patients inadequately controlled on insulin glargine, insulin detemir, or NPH insulin received either linagliptin 5 mg once daily ( $n=126$ ) or placebo ( $n=121$ ). Mean insulin doses were 35.0 IU and 36.6 IU, respectively. Baseline age and HbA1c were 74.3 years and 8.2% in both groups. Overall, 95.1% of all individuals had a diabetes duration  $>5$  years. At week 24, the placebo-adjusted mean change in HbA1c with linagliptin was  $-0.77\%$  (95% CI:  $-0.95$ ,  $-0.59$ ;  $P < 0.0001$ ). AE rates were generally lower with linagliptin than placebo. The overall incidence of AEs was 75.4% with linagliptin and 81.0% with placebo. Drug-related AE rates were 16.7% with linagliptin and 20.7% with placebo. The majority of AEs with either linagliptin or placebo were mild-to-moderate in intensity (severe AE rates: 5.6% vs. 7.4%, respectively) and most were non-serious in nature (serious AE rates: 9.5% vs. 18.2%, respectively). Hypoglycaemia occurred in 28.6% on linagliptin and 37.2% on placebo. The frequency of premature discontinuation was lower with linagliptin than with placebo (7.1% vs. 14.0%, respectively). The most common reasons for occurrence of an AE (4.0% on linagliptin vs. 3.3% on placebo) and refusal to continue trial medication (1.6% on linagliptin vs. 5.0% on placebo).

**Conclusion:** In this vulnerable elderly population, linagliptin in combination with basal insulin was well tolerated and achieved clinically meaningful improvements in glycaemic control without excessive risk of hypoglycaemia.

Clinical Trial Registration Number: NCT00954447 & NCT01084005

Supported by: Boehringer Ingelheim

### 849

#### Efficacy and safety of linagliptin as add-on therapy to sulphonylurea (SU) in type 2 diabetes patients with moderate or severe renal impairment (RI)

M. von Eynatten<sup>1</sup>, A.H. Barnett<sup>2</sup>, S. Patel<sup>3</sup>, D. Neubacher<sup>4</sup>, H.-J. Woerle<sup>1</sup>;

<sup>1</sup>Boehringer Ingelheim, Ingelheim, Germany, <sup>2</sup>University of Birmingham and Heart of England NHS Foundation Trust, Birmingham, UK, <sup>3</sup>Boehringer Ingelheim, Bracknell, UK, <sup>4</sup>Boehringer Ingelheim, Biberach, Germany.

**Background and aims:** Since metformin is not recommended in patients with type 2 diabetes mellitus (T2DM) and more advanced forms of RI, combination therapy with a DPP-4 inhibitor and a SU may be a viable alternative. In contrast to other DPP-4 inhibitors, linagliptin has a primarily non-renal route of excretion and a one-strength, once-daily dose. We evaluated the efficacy and safety of linagliptin as add-on to SU in T2DM patients with moderate or severe RI based on estimated GFR.

**Materials and methods:** A post-hoc analysis was conducted that included data from three Phase 3, randomised, double-blind, placebo-controlled studies of linagliptin 5 mg once daily for treatment of T2DM. Only patients in which linagliptin was added to background SU were eligible for the analysis.

Study 1 was conducted over 24 weeks in elderly patients ( $\geq 70$  years;  $\pm$  insulin  $\pm$  oral antidiabetes background therapy), Study 2 over 18 weeks in patients with insufficient glycaemic control on SU monotherapy, and Study 3 over 12 weeks (primary efficacy endpoint; full study was 52 weeks) in patients with severe chronic RI ( $\pm$  insulin  $\pm$  oral antidiabetes background therapy).

**Results:** Across the studies, 91 patients were identified with moderate or severe RI who had previously received SU; of these 58 received linagliptin and 33 received placebo. Baseline values for linagliptin versus placebo were: age (SD), 69 (10.2) vs 70 (8.3) years; male sex, 67% vs 55%; and HbA1c (SD), 8.2% (1.1) vs 8.1% (0.6). Overall, 91.2% of patients had diabetes duration  $> 5$  years and 62.6% were receiving  $\geq 2$  antidiabetes drugs at enrolment. Significantly greater ( $P < 0.05$ ) decreases in HbA1c compared with placebo (adjusted mean [SE]) were observed in Study 1 ( $-0.68\%$  [0.25]) and 2 ( $-1.08\%$  [0.43]), and a trend for a numerically greater decrease in Study 3 ( $-0.62\%$  [0.31];  $P = 0.06$ ) (Table). Across all studies, adverse events (AEs) and serious AEs were similar between linagliptin and placebo: 46 (79.3%) and 13 (22.4%) compared with 25 (75.8%) and 9 (27.3%), respectively. The overall incidence of hypoglycaemia was slightly lower in patients on linagliptin (22/58; 37.9%) compared with placebo (13/33; 39.4%). Hypoglycaemic events with linagliptin were mostly of mild intensity; 12 patients (20.7%) experienced a mild episode while only 1 patient (1.7%) experienced a severe episode. There was no difference between the two groups with regards to changes in body weight in any of the studies.

**Conclusion:** In patients with moderate or severe RI, linagliptin as an add-on treatment to SU resulted in clinically important reductions in HbA1c without an unacceptable risk of AEs. In particular, the incidence of hypoglycaemia was not further increased with linagliptin. Our data support the use of linagliptin as add-on to SU in this difficult to treat population in which treatment alternatives are scarce.

Table							
Study (duration)	Treatment	N	Baseline mean (SD)	Adjusted mean change from baseline (SE)	Difference to placebo		
					Adjusted mean (SE)	95% CI	P-value
HbA1c, %							
1 (24 weeks)	Placebo	10	7.77 (0.60)	-0.05 (0.23)	-0.68 (0.25)	-1.19, -0.17	0.01
	Linagliptin	26	7.96 (0.76)	-0.73 (0.14)			
2 (18 weeks)	Placebo	8	8.34 (0.57)	0.29 (0.31)	-1.08 (0.43)	-2.02, -0.14	0.03
	Linagliptin	9	8.62 (0.48)	-0.79 (0.29)			
3 (12 weeks)	Placebo	15	8.15 (0.56)	-0.25 (0.24)	-0.62 (0.31)	-1.25, 0.01	0.06
	Linagliptin	23	8.23 (1.45)	-0.86 (0.19)			

CI, confidence interval; SD, standard deviation; SE, standard error

Clinical Trial Registration Number: NCT00819091, NCT00800683, NCT01084005

Supported by: Boehringer Ingelheim

## 850

### Safety and efficacy of linagliptin in elderly patients with type 2 diabetes: evidence from 1331 individuals aged $\geq 65$ years

S. Patel<sup>1</sup>, G. Scherthaner<sup>2</sup>, A.H. Barnett<sup>3</sup>, A. Emser<sup>4</sup>, M. von Eynatten<sup>4</sup>, H.-J. Woerle<sup>4</sup>;

<sup>1</sup>Boehringer Ingelheim Ltd, Bracknell, UK, <sup>2</sup>Department of Medicine I Rudolfstiftung Hospital, Vienna, Austria, <sup>3</sup>University of Birmingham and Heart of England NHS Foundation Trust, Birmingham, UK, <sup>4</sup>Boehringer Ingelheim, Ingelheim, Germany.

**Background and aims:** The treatment of type 2 diabetes mellitus (T2DM) in elderly patients can be challenging because of safety and tolerability concerns, such as associated co-morbidities and higher risk of treatment-related complications, including hypoglycaemia and gastrointestinal (GI) side-effects. Using pooled data from the large, international linagliptin Phase 3 program, we investigated the safety and efficacy of linagliptin in elderly patients.

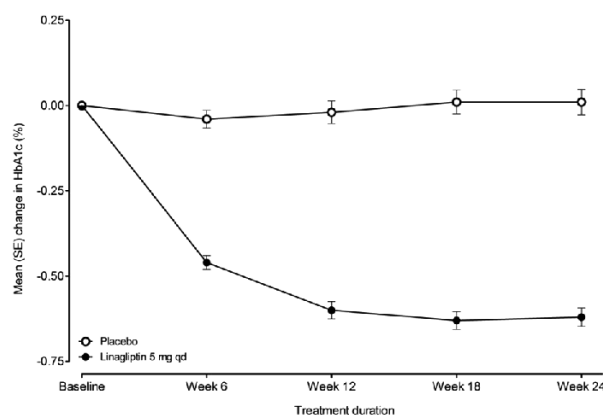
**Materials and methods:** Data ( $n = 1331$ ) were pooled from 7 randomised, double-blind, placebo-controlled Phase 3 trials of linagliptin 5 mg once daily as monotherapy or add-on to various glucose-lowering therapies of at least 24 weeks duration. This post-hoc sub-analysis comprised all patients aged  $\geq 65$  years. Safety and efficacy parameters for this analysis were assessed up to week 24.

**Results:** In total, 841 patients received linagliptin and 490 received placebo. Mean (SD) baseline characteristics were similar in the linagliptin vs placebo

groups: age, 71.1 (4.5) vs 70.9 (4.7) years; BMI, 29.5 (5.0) vs 30.0 (4.9) kg/m<sup>2</sup>; HbA1c, 8.0% (0.8) vs 8.1% (0.8). Overall, 21% of patients had renal function decline (eGFR  $< 60$  mL/min), more than 80% of patients had diabetes duration  $> 5$  years and more than 60% of patients were receiving  $\geq 2$  glucose-lowering drugs. Median exposure to linagliptin and placebo was 173.0 and 176.5 days, respectively. The placebo-adjusted mean (95% CI) change in HbA1c from baseline to week 24 was  $-0.62\%$  ( $-0.73, -0.51$ ;  $P < 0.0001$ ) for patients who received linagliptin (Figure). A significantly greater reduction in fasting plasma glucose (FPG) from baseline to week 24 was noted for linagliptin (placebo-adjusted mean [95% CI] change:  $-14.8$  mg/dL [ $-20.7, -8.9$ ];  $P < 0.0001$ ;  $-0.82$  mmol/L [ $-1.15, -0.49$ ]). Adverse events (AEs) were experienced by 71.3% and 73.3% of patients who received linagliptin and placebo, respectively, and drug-related AEs were less frequent with linagliptin treatment (18.1%) than with placebo (19.8%). The incidence of hypoglycaemia was slightly lower in patients who received linagliptin (21.4%) compared with placebo (25.7%). Severe hypoglycaemic events requiring assistance were rare in both groups (1.0% and 1.8%, respectively). Overall AE reporting for GI disorders was comparable between both groups (14.1% and 15.5%, respectively). At least one adjudicated cardiovascular event was experienced by 0.8% and 1.0% of linagliptin- and placebo-treated patients, respectively.

**Conclusion:** Although this patient population is frequently compromised in renal function and is at high risk for hypoglycaemia, linagliptin is well tolerated and an efficacious treatment option in elderly patients without the need for dose adjustment.

**Figure:** Mean change in HbA1c over time. Full analysis set (LOCF analysis)



Supported by: Boehringer Ingelheim

## 851

### Efficacy and safety of gemigliptin in patients with type 2 diabetes

S.J. Yang<sup>1</sup>, K.W. Min<sup>2</sup>, J.Y. Park<sup>3</sup>, D.M. Kim<sup>4</sup>, Y.S. Kim<sup>5</sup>, S.K. Gupta<sup>6</sup>, V.K. Shivane<sup>7</sup>, S. Pitale<sup>8</sup>, P.K. Agarwal<sup>9</sup>, A.R. Sosale<sup>10</sup>, M. Dharmalingam<sup>11</sup>, P. Gandhi<sup>12</sup>, V. Mohan<sup>13</sup>, J.A. Kim<sup>14</sup>, S.H. Baik<sup>1</sup>;

<sup>1</sup>Korea University Guro Hospital, Republic of Korea, <sup>2</sup>Eulji Medical Center Medical Corporation, Seoul, Republic of Korea, <sup>3</sup>Asan Medical Center, Asan Public Welfare Foundation, Seoul, Republic of Korea, <sup>4</sup>Kangdong Sacred Heart Hospital, Seoul, Republic of Korea, <sup>5</sup>Inha University Hospital, Incheon, Republic of Korea, <sup>6</sup>MV Hospital and Research Centre, Lucknow, India, <sup>7</sup>RHIDEM, Mumbai, India, <sup>8</sup>Pitale Diabetes and Hormone Center, Nagpur, India, <sup>9</sup>Hormone care and research centre, Ghaziabad, India, <sup>10</sup>Diacon Hospital, Diabetes Care and Research Centre, Bangalore, India, <sup>11</sup>Bangalore Endocrinology and Diabetes Research Centre, Bangalore, India, <sup>12</sup>Gandhi Research Institute, Nagpur, India, <sup>13</sup>Madras Diabetes Research Foundation, Chennai, India, <sup>14</sup>LG Life Sciences, Seoul, Republic of Korea.

**Background and aims:** This study was designed to assess the efficacy and safety of a DPP IV inhibitor, gemigliptin (LC15-0444) 50mg versus placebo in patients with type 2 diabetes.

**Materials and methods:** We conducted a 24-week, randomized, double-blind, placebo-controlled phase III trial in 182 patients (74 from Korea and 108 from India) with type 2 diabetes. After initial 2 weeks of exercise/diet program followed by another 2 weeks of single-blind placebo run-in period, eligible patients were randomized to gemigliptin 50mg or placebo group and received the assigned treatment for 24 weeks. HbA1c and fasting plasma glucose (FPG) were measured periodically, and oral glucose tolerance tests were performed at baseline, week 12 and week 24.



**Results:** At week 24, gemigliptin treatment led to significant reductions in HbA<sub>1c</sub> compared with placebo in HbA<sub>1c</sub> (-0.71%). A significantly greater proportion of patients achieved an HbA<sub>1c</sub> <7% with gemigliptin than with placebo. The placebo-subtracted FPG change from baseline at week 24 was -19.80 mg/dL. The overall incidence rate of adverse events in the gemigliptin and in the placebo was similar between the two groups.

**Conclusion:** In conclusion, this study demonstrated the efficacy and safety of gemigliptin 50mg administered once daily as a monotherapy in type 2 diabetes patients.

Supported by: LG Life Sciences

## 852

### Efficacy and safety of gemigliptin compared with sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone

E.J. Rhee<sup>1</sup>, K.W. Min<sup>2</sup>, H.C. Jang<sup>3</sup>, C.H. Chung<sup>4</sup>, I.S. Nam-Goon<sup>5</sup>, H.Y. Bae<sup>6</sup>, M.K. Lee<sup>7</sup>, S.H. Baik<sup>8</sup>, V.K. Shivane<sup>9</sup>, A.R. Sosale<sup>10</sup>, M. Dharmalingam<sup>11</sup>, P. Gandhi<sup>12</sup>, S.K. Gupta<sup>13</sup>, J.A. Kim<sup>14</sup>, S.W. Kim<sup>1</sup>;

<sup>1</sup>Kangbuk Samsung Medical Center, Seoul, Republic of Korea, <sup>2</sup>Eulji Medical Center Medical Corporation, Seoul, Republic of Korea, <sup>3</sup>Seoul National University Bundang Hospital, Gyeonggi, Republic of Korea, <sup>4</sup>Wonju Christian Hospital, Gangwon, Republic of Korea, <sup>5</sup>Ulsan University Hospital, Ulsan, Republic of Korea, <sup>6</sup>Chosun University Hospital, Gwangju, Republic of Korea, <sup>7</sup>Samsung Medical Center, Seoul, Republic of Korea, <sup>8</sup>Korea University Guro Hospital, Seoul, Republic of Korea, <sup>9</sup>RHIDEM, Mumbai, India, <sup>10</sup>Diacon Hospital, Diabetes Care and Research Centre, Bangalore, India, <sup>11</sup>Bangalore Endocrinology and Diabetes Research Centre, Bangalore, India, <sup>12</sup>Gandhi Research Institute, Nagpur, India, <sup>13</sup>M V Hospital and Research Centre, Lucknow, India, <sup>14</sup>LG Life Sciences, Seoul, Republic of Korea

**Background and aims:** This study was designed to assess the efficacy and safety of a DPP IV inhibitor, gemigliptin (LC15-0444) 50mg versus sitagliptin 100mg in patients with type 2 diabetes continuing metformin treatment.

**Materials and methods:** We conducted a 24-week, double-blind, randomized, active-controlled trial in 425 patients (296 from Korea, 129 from India) with type 2 diabetes inadequately controlled with metformin alone. Eligible patients were randomized to one of 3 treatment groups and gemigliptin 50mg qd or gemigliptin 25mg bid or sitagliptin 100mg qd was added to ongoing metformin treatment for 24 weeks. HbA<sub>1c</sub> and fasting plasma glucose (FPG) were measured periodically, and oral glucose tolerance tests were performed at baseline, and week 24.

**Results:** At week 24, gemigliptin 50mg/day added to ongoing metformin therapy resulted significant improvement of glycemic control supported by the followings. Reduction in HbA<sub>1c</sub> of gemigliptin 50mg qd (-0.77%) was non-inferior to those once daily sitagliptin 100mg (-0.83%). Proportion of patients achieving HbA<sub>1c</sub> <7% or 6.5% in gemigliptin 25mg bid (50%) and gemigliptin 50mg qd (54.07%) was comparable to the results with once daily sitagliptin 100 mg (48.87%). There was decrease in FPG, postprandial glucose, AUC<sub>0-2h</sub> glucose, DPP 4 activity and increase in GLP-1, sensitivity of beta cells to glucose (supported by HOMA-β, HOMA-IR, postprandial (2h) c-peptide, insulinogenic index, and postprandial proinsulin to insulin ratio) in patients who were administered with gemigliptin 50mg/day added to ongoing metformin therapy. There was no increased risk of adverse experience with gemigliptin 50mg/day compared with sitagliptin 100mg qd.

**Conclusion:** In conclusion, gemigliptin 50mg/day as an add-on therapy to metformin was efficacious and well tolerated in type 2 diabetes mellitus patients.

Supported by: LG Life Sciences

## 853

### DPP-4 inhibitor vildagliptin reduces postprandial RLP-cholesterol and RLP-triglycerides in type 2 diabetes

N. Matikainen, M.-R. Taskinen;

Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland.

**Background and aims:** Pharmacological inhibition of DPP-4 has been shown to reduce triglyceride (TG)-rich lipoproteins in experimental studies, suggesting that DPP-4 inhibitors may improve postprandial diabetic dyslipidemia. If Vildagliptin (VILDA) reduces postprandial atherogenic remnant

particles is unknown. Therefore, we assessed the effects of VILDA on remnant like-particle cholesterol (RLP-Chol), RLP-TG, apolipoprotein (apo) A5, and apoCIII concentrations in a randomized clinical trial in subjects with T2DM.

**Materials and methods:** Current data represent a substudy of our previous study with twenty-three drug-naïve T2DM patients (age 39-69 years), who participated in this single-centre, randomized, double-blind, placebo-controlled, parallel-group study of 4-week duration. Entry criteria included HbA<sub>1c</sub> of 6.5-10.0%, fasting plasma glucose of <13.3 mmol/L, fasting TG of 1.7-5.0 mmol/L and BMI of 25-40 kg/m<sup>2</sup>. Subjects with an apo E2-allele or lipid-lowering therapy within 3 months prior to screening were excluded from the study. We measured RLP-cholesterol, RLP-TG, apoA5 and apoCIII before and 3, 4, 6 and 8 hrs after an oral fat load (72 g fat, 50 g carbohydrates, 35 g protein) at week 0 and 4 during VILDA 50 mg bid.

**Results:** At baseline, subjects receiving VILDA (n=11) and PBO (n=12) had comparable age, BMI, glycemic control and fasting TG. Treatment with VILDA produced significant reduction by -35.4 mg/dL\*h (p=0.035), in postprandial RLP-cholesterol area under the curve (AUC) from week 0 to 4, while no change occurred with PBO (RLP-cholesterol AUC change +5.2 mg/dL\*h, NS). Plasma TG AUC decreased in VILDA group, but not in PBO group. In accordance, RLP-TG AUC decreased by -339 mg/dL\*h with VILDA (p=0.011) from week 0 to 4 but remained unchanged with PBO (+121 mg/dL\*h, NS). Furthermore, treatment with VILDA produced borderline-significant reduction in postprandial apoA5 AUC from week 0 to 4 (p=0.082 for VILDA, NS for PBO). We detected no changes in postprandial apoCIII AUC in either group. HbA<sub>1c</sub> at baseline averaged 6.9±0.7% and 6.8±0.7% in patients treated with VILDA and PBO, respectively. In patients randomized to VILDA HbA<sub>1c</sub> decreased by 0.5% from week 0 to 4 (p<0.01), while it remained unchanged in PBO group. **Conclusion:** Treatment with VILDA reduces postprandial RLP-cholesterol and RLP-TG i.e. atherogenic RLP remnant particles. These changes may contribute to an improved cardiovascular risk profile in patients with T2DM.

Clinical Trial Registration Number: NCT00099957

Supported by: Novartis

## 854

### Persistence of treatment with DPP-4 inhibitors and sulfonylureas in primary care practices in Germany and France: a retrospective analysis

J. Rex<sup>1</sup>, K. Kostev<sup>1</sup>, J.-B. Gruenberger<sup>2</sup>, G. Bader<sup>2</sup>, M. Dworak<sup>3</sup>, W. Rathmann<sup>4</sup>, G. Giani<sup>4</sup>;

<sup>1</sup>TMS Health, Epidemiology, Frankfurt, <sup>2</sup>Novartis Pharma AG, Basel, Switzerland, <sup>3</sup>Novartis Pharma GmbH, Nuernberg, <sup>4</sup>Institute of Biometrics and Epidemiology, Düsseldorf, Germany.

**Background and aims:** Persistence with medications is essential for therapeutic success in chronic diseases such as type 2 diabetes mellitus (T2DM). The persistence, i.e. the extent to which patients continue treatments and regularly take their medications, was compared for dipeptidyl peptidase-4 inhibitors (DPP-4s) and sulfonylureas (SUs) in primary care practices in Germany and France.

**Materials and methods:** Non-persistence was defined as a period of ≥90 days without prescriptions (DPP-4s, SUs). Longer intervals were considered as treatment gaps and the anti-diabetic therapy of patients was classified as no longer persistent (maximum follow-up duration: 2 years). New prescriptions (index date) of DPP-4 and SU (ATC codes) in patients with T2DM without additional anti-diabetic agents except for metformin in primary care practices in Germany (n=1,201 physicians) and in France (n=682 physicians) were retrospectively analysed (Disease Analyser database, 04/2007-08/2011). The lack of persistence (DPP-4s vs. SUs) was compared using Cox regression models after adjusting for age, sex, diabetes duration, episodes of hypoglycaemia, health insurance (private), practice region, and Charlson Comorbidity Index.

**Results:** After two years, 61% of patients treated with DPP-4s (n=17,312) and only 51% of patients treated with SUs (n=30,382) in German practices persisted with their anti-diabetic treatment. Similar results were found in France where 59% of patients treated with DPP-4s (n=8,675) vs. 51% with SUs (n=11,150) persisted with their treatments. In the adjusted Cox model, prescriptions of DPP-4s compared to SUs were associated with a lower risk of discontinuation of therapy in Germany (hazard ratio: 0.74 [95% CI: 0.71, 0.76]; p<0.001). A similarly reduced risk of discontinuation was found in patients in France (HR: 0.79 [95% CI: 0.75, 0.83]; p<0.001).

**Conclusion:** Lack of persistence with anti-diabetic therapy is a common problem in patients with T2DM treated in primary care practices in Germany and France. A larger proportion of patients on DPP-4 inhibitors persisted with

treatment compared to sulfonylureas. The potential reasons for this difference including hypoglycaemia and weight gain must be investigated in further studies.

Supported by: Novartis Pharma

## 855

### Clinical outcome of treatment with DPP-4-inhibitors or GLP-1-analogues in patients with type 2 diabetes treated in diabetes specialised medical practices in Germany

G. Hess, winDiab;  
winDiab, Düsseldorf, Germany.

**Background and aims:** In many patients with Type 2 diabetes treated in daily life in Diabetes Specialized Medical Practices (DSP) a treatment with a DPP-4-inhibitor (DPP-4) or GLP-1-analog (GLP-1) is started. We analysed the outcome of this treatment in 907 patients treated in 38 DSPs for a maximal period of 1 year with respect to factors that have an impact on the success of incretin-based treatment in an observational study.

**Materials and methods:** After the decision for an incretin based therapy was made in a mutual conclusion between the patient and the diabetologist they were included in this study independent of their pre-treatment. A number of parameters was documented at start of therapy and was evaluated again after 3, 6 and 12 months (also if treatment with an incretin-based drug was stopped before). The patients were analysed according to the incretin drug they were treated with: DPP-4: 437 patients, female 44.4%, age 59±12 years, duration of diabetes 8.4±6.7 years, HbA1c 8.4±1.5%, BMI 34.2±6.9 kg/m<sup>2</sup>; GLP-1: 470 patients, female 50.2%, 56±10 years, 9.1±5.8 years, 8.4±1.4%, 39.3±7.2 kg/m<sup>2</sup>. These groups were further subdivided in the analysis according to pre-established insulin therapy.

**Results:** In both groups one drug was used most often: DPP-4: Sitagliptin n= 327 patients (75%; related to the 437 patients/100% in this group), Vildagliptin 76 (17%), Saxagliptin 34 (8%); GLP-1: Liraglutid 402 (86%; related to the 470 patients in this group), Exenatide 68 (14%). From those treated with DPP-4 223 patients (51%) were still treated with such a drug after 12 months, from those treated with GLP-1 262 (56%). In the other patients treatment was stopped (105, 24% vs. 142, 30%) or they did not come back to the follow-up visits (109, 24% vs. 66, 14%). Main reasons for stopping treatment were: Insufficient effect (60, 57% of 105 vs. 115, 81% of 109), side effects (24, 23% vs. 28; 20%) or patient's wish (27, 26% vs. 18, 13%). Treatment with a DPP-4 for 12 months induced a reduction in BMI by 0.4 kg/m<sup>2</sup> (median difference; from 32.8 to 32.2 kg/m<sup>2</sup> (median)); with GLP-1 the decline was 1.5 kg/m<sup>2</sup> (from 39.1 to 37.2 kg/m<sup>2</sup>; p<0.001). HbA1c was lowered by 0.6% (from 7.8% to 7.2%) by DPP-4 and by 0.1% (from 8.2% to 7.8%) in patients treated with GLP-1 that were previously treated with insulin and by 1.1% (from 8.3% to 7.1%; p<0.001) in those without previous insulin. A reduction in HbA1c by more than 1.0% was observed in 61 (37%) of 164 patients treated with DPP-4 without insulin at start of therapy and in 17 (29%) of 59 with insulin. In the patients treated with GLP-1 such a reduction was observed in 70 (51%) of 137 patients without insulin at start, but in only 28 (22%) of the 125 with insulin.

**Conclusion:** In a considerable number of patients treated with either of these new drugs the treatment ended within 12 months despite being treated in a DSP. GLP-1 induced a more pronounced improvement in metabolic control (depending on the therapy before) and also lowering of body weight as DPP-4 (starting at different levels). However, in individual patients a considerable improvement in both parameters (metabolic control and body weight) was observed without having clear predictive parameters for treatment success.

## PS 068 DPP-4 inhibitors II

### 856

#### Vildagliptin efficacy and safety in patients with type 2 diabetes inadequately controlled on dual metformin plus sulfonylurea therapy

V. Lukashevich<sup>1</sup>, M. Wang<sup>1</sup>, S. Del Prato<sup>2</sup>, M. Araga<sup>3</sup>, W. Kothny<sup>1</sup>;

<sup>1</sup>Novartis Pharmaceutical Corporation, East Hanover, USA, <sup>2</sup>University of Pisa, Italy, <sup>3</sup>Novartis Pharma AG, Basel, Switzerland.

**Background and aims:** The combination of metformin (MET) plus a sulfonylurea (SU) is broadly used for the treatment of type 2 diabetes (T2DM). However, many patients on dual therapy still do not achieve glycaemic control. Adding a third oral agent can be an option for patients before initiating insulin therapy. Vildagliptin (VILDA), a potent and selective dipeptidyl peptidase-4 inhibitor with proven efficacy with either MET or an SU, was assessed in such a triple therapy setting.

**Materials and methods:** The efficacy and safety of VILDA 50 mg bid as add-on therapy to a stable dose of MET (≥ 1500 mg) plus glimepiride (≥ 4 mg) was assessed in a multi-center, double-blind, randomised, placebo-controlled 24-week study in 318 T2DM patients (N=158 on VILDA and N= 160 on placebo) with inadequate glycaemic control (HbA1c ≥7.5 and ≤11%). The HbA1c change versus placebo was the primary study endpoint.

**Results:** The study population had a mean age of 55.1 years, mean T2DM duration of 7.3 years, mean BMI of 28.0 kg/m<sup>2</sup>, mean fasting plasma glucose (FPG) of 9.4 mmol/L and mean HbA1c of 8.8%. VILDA demonstrated a clinically relevant and statistically significant reduction in HbA1c compared to placebo (Table 1). Approximately 30% of VILDA-treated patients reached HbA1c target <7% vs. 6% in the placebo group (p<0.001). The difference in FPG reduction between VILDA and placebo of 1.13 mmol/L (baseline FPG 9.34 mmol/L and 9.52 mmol/L, respectively) was also clinically and statistically significant (p<0.001). VILDA was well tolerated with an overall safety profile similar to placebo. The rate of hypoglycaemia was overall low but slightly higher with VILDA than with placebo (5.1% vs. 1.9%). There was no clinically relevant weight gain with VILDA.

**Conclusion:** VILDA 50 mg bid demonstrated robust glucose-lowering efficacy in patients with T2DM inadequately controlled on dual metformin plus glimepiride therapy. The triple combination with VILDA was safe and well tolerated with low risk of hypoglycaemia and weight gain. This makes vildagliptin an attractive treatment addition for patients failing on dual metformin and SU therapy.

Table 1: ANCOVA results for change in HbA1c (%) from baseline to endpoint\* by treatment (Full Analysis Set)

				Difference in adjusted mean change (VILDA-Placebo)		
Treatment	n	Baseline mean (SE)	Adjusted mean change Mean (SE)	Mean (SE)	( 95% CI )	P-Value
Full analysis Set						
VILDA 50mg bid + MET + GLIM	152	8.75 (0.07)	-1.01 (0.09)	-0.76 (0.12)	(-0.98, -0.53)	<0.001
Placebo + MET + GLIM	160	8.80 (0.07)	-0.25 (0.09)			

\* censored by the start of rescue medication use

GLIM - glimepiride; VILDA - vildagliptin; MET - metformin; ANCOVA - Analysis of covariance

Clinical Trial Registration Number: NCT01233622

Supported by: Novartis Pharma

## 857

# Vildagliptin added to once or twice daily insulin regimens improves glycaemic control without increasing risk of hypoglycaemia and weight gain in patients with type 2 diabetes

W. Kothny<sup>1</sup>, P. Kozlovski<sup>2</sup>, J. Foley<sup>1</sup>, Q. Shao<sup>1</sup>, V. Lukashevich<sup>1</sup>

<sup>1</sup>Novartis Pharmaceutical Corporation, East Hanover, USA, <sup>2</sup>Novartis Pharma AG, Basel, Switzerland.

**Background and aims:** Vildagliptin (Vilda) blocks dipeptidyl peptidase-4 (DPP-4) inhibition of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) which extends their physiological effects, thereby increasing islet alpha- and beta-cell responsiveness to glucose. While both GLP-1 and GIP enhance glucose mediated insulin (INS) secretion GLP-1 reduces glucagon levels in hyperglycaemia and GIP increases glucagon in hypoglycaemia. We previously reported that Vilda 50 mg bid added to INS monotherapy in patients with type 2 diabetes mellitus (T2DM) provided 0.3% reduction in HbA1c vs. placebo (Pbo) with reduced risk of hypoglycaemia. The efficacy results were confounded by a high dose INS (>80 U/day) regimen with significant short acting INS usage suppressing endogenous insulin secretion. We now report a 24-week study to assess the efficacy and safety of Vilda in patients with T2DM inadequately controlled by stable basal, intermediate acting, or pre-mixed INS, with or without concomitant metformin, which is considered to be more reflective of current practice than the previous trial.

**Materials and methods:** In a 24-week, multicentre, randomised, double-blind, parallel-group, placebo controlled study 449 patients with T2DM on stable insulin therapy with long-acting, intermediate-acting or pre-mixed insulin were randomized to vildagliptin 50 mg bid (N=228) and placebo (N=221). Stable metformin background therapy was permitted. The primary endpoint was efficacy expressed as change in HbA1c versus placebo. Safety and tolerability were also evaluated.

**Results:** Patients in this trial were 59 years old with a T2DM duration of 13 years. 60.6% of patients were on premixed INS regimens, 23% on long-acting and 16.4% on intermediate acting INS. The mean dose of INS was 40.9 U/day and approximately 60% of patients were on stable metformin background therapy. Vilda significantly reduced HbA1c compared to Pbo in the overall population and sub-groups with or without metformin (Table 1). Overall Vilda was safe and well tolerated with no weight increase. Incidences of hypoglycaemia (8.4% vs 7.2%) and severe hypoglycaemia events (0.9% in both groups) were similarly low in Vilda and Pbo patients respectively despite the substantially greater HbA1c reduction with Vilda.

**Conclusion:** Usually increased hypoglycaemia limits the efficacy of oral hypoglycaemic agents in combination with INS. Vilda not only significantly lowered HbA1c without weight gain, but also did not increase hypoglycaemia incidence presumably due to a GIP mediated counter-regulatory glucagon effect. Thus, Vilda is an attractive combination partner with INS, with or without concomitant metformin therapy.

Table 1. ANCOVA results for change in HbA1c (%) from BL to study EP censored by rescue medication

				Difference in adjusted mean change (Vilda-Placebo)		
Treatment	n	Baseline mean (SE)	Adjusted mean change (SE)	Mean (SE)	( 95% CI )	P-Value
Full-analysis set						
Vilda 50mg bid	221	8.80 (0.07)	-0.77 (0.08)	-0.72 (0.10)	(-0.92, -0.52)	<0.001*
Placebo	215	8.84 (0.07)	-0.05 (0.08)			
Full-analysis set (Insulin + metformin)						
Vilda 50mg bid	133	8.78 (0.08)	-0.98 (0.09)	-0.63 (0.12)	(-0.86, -0.39)	<0.001*
Placebo	134	8.80 (0.08)	-0.35 (0.09)			
Full-analysis set (Insulin)						
Vilda 50mg bid	88	8.84 (0.12)	-0.60 (0.19)	-0.84 (0.19)	(-1.21, -0.47)	<0.001*
Placebo	81	8.90 (0.11)	0.24 (0.20)			
ANCOVA - Analysis of covariance; BL - Baseline; EP - Endpoint; SE - Standard error; Vilda - Vildagliptin						

ANCOVA - Analysis of covariance; BL - Baseline; EP - Endpoint; SE - Standard error;

Vilda - Vildagliptin

Clinical Trial Registration Number: NCT01224366

Supported by: Novartis Pharma

## 858

# Vildagliptin as add-on therapy to other oral antidiabetics in Japanese patients with type 2 diabetes

M. Suzuki<sup>1</sup>, I. Hamada<sup>2</sup>, M. Odawara<sup>3</sup>, LAF237A1308 Study Group;

<sup>1</sup>Primary Care Clinical Franchise Dept., Novartis Pharma KK, <sup>2</sup>IHC/RESP IIS Franchise Dept., Novartis Pharma KK, <sup>3</sup>The Third Department of Internal Medicine, Tokyo Medical University, Japan.

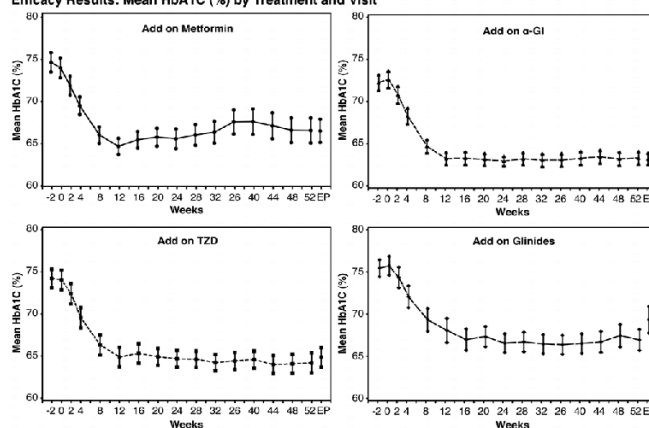
**Background and aims:** We report efficacy results from a long-term (52-week) safety and tolerability study in Japanese patients with vildagliptin as add-on therapy to approved oral antidiabetic drugs (OADs) including metformin, thiazolidinediones (TZD), alpha-glucosidase inhibitors (α-GI), or glinides. Efficacy variables included change from baseline to endpoint (week 52 or final study visit) in HbA1c and fasting plasma glucose (FPG), while safety analyses compared the incidence of adverse events (AEs) during the treatment period.

**Materials and methods:** Two hundred and forty five eligible patients (vildagliptin + metformin, n=58; vildagliptin + TZD, n=62; vildagliptin + α-GI, n=62; vildagliptin + glinide, n=63), uncontrolled on monotherapy, were included in this 52-week, multicentre, open-label study. All enrolled patients were included in the Safety and Full Analysis Set populations.

**Results:** Treatment groups were well-balanced for baseline demographic and background characteristics. Mean age of patients was 59.5 years (~65% were <65 years old). Mean baseline values of HbA1c (JDS unit), FPG and BMI were 7.41%, 161.3 mg/dL and 25.6 kg/m<sup>2</sup> respectively. Mean duration of diabetes was 6.5 years. Of the enrolled patients, 86.1% completed the study. Mean change in HbA1c was -0.81% (95% CI -0.91, -0.71). Overall, 62.2% patients achieved HbA1c <7.0% and 71.4% had HbA1c improved by ≥0.5% at endpoint. The figure shows change in mean HbA1c in each group over time. HbA1c change was lowest in the glinide group (-0.64% [95% CI -0.85, -0.43]) and highest in the α-GI group (-0.94% [95% CI -1.15, -0.74]). Furthermore, the glinide group had lower HOMA-B (25.663) and C-peptide (1.38 ng/mL), and higher HOMA-IR levels (3.259) at baseline than other groups. The percentage of patients reporting an AE (≥3%) was comparable in all groups; 86.5% experienced at least one AE. Infections (50.6%) were the most frequently occurring pre-defined risk, of which nasopharyngitis (30.6%) was most frequently reported. All cases of infections were mild or moderate. Overall 19 serious AEs occurred in 15 patients (6.1%). No deaths were reported. Percentage of patients discontinuing from the study was more in the glinide group (23.8%) than other groups (6.5%-16.1%). One patient experienced mild hypoglycaemia in the metformin add-on group.

**Conclusion:** Vildagliptin (50 mg bid) was generally safe, well tolerated, and resulted in clinically significant reduction in HbA1c across treatment groups, when used as add-on therapy to other OADs in patients with T2DM, in a first of its kind study in Japan.

Efficacy Results: Mean HbA1C (%) by Treatment and Visit



Unadjusted means and standard errors (vertical bars) are given. Baseline measurement is obtained on Day 0 or sample obtained on an earlier visit (scheduled/unscheduled) closest to Day 0, if Day 0 measurement is missing. EP (Endpoint) is the final available post-baseline assessment up to the last regular scheduled visit. HbA1c is reported in JDS unit (%). HbA1c (NGSP, %) = HbA1c (JDS, %) + 0.4.

Clinical Trial Registration Number: NCT01159249

Supported by: Novartis Pharma



## 859

**Vildagliptin improves a chronic effect on beta cell function when added to insulin therapy**J. Foley<sup>1</sup>, P. Kozlovski<sup>2</sup>, V. Lukashevich<sup>1</sup>, W. Kothny<sup>1</sup>;<sup>1</sup>Novartis Pharmaceuticals Corporation, East Hanover, USA, <sup>2</sup>Novartis Pharma AG, Basel, Switzerland.

**Background and aims:** Vildagliptin increases glucose (GLU) sensitive insulin (INS) secretion and decreases INS sensitive glucagon secretion; these effects are considered acute (i.e. direct drug effects). In a 24-week randomized, double-blind, placebo-controlled study, vildagliptin 50mg bid showed significant improvement in glycemic control in patients with type 2 diabetes inadequately controlled on stable dose of basal or premixed INS, with or without concomitant metformin (MET) treatment compared to placebo. One of the study objectives was to evaluate the chronic effect of vildagliptin on beta-cell function and INS sensitivity in this patient population; after 24 weeks of treatment these parameters were measured in the absence of INS, vildagliptin and MET.

**Materials and methods:** GLU and INS response were assessed during a 2 hour OGTT performed at baseline, and after 24 weeks of treatment. Patients did not take their study medication, INS injection, and MET (if applicable) on the morning of the OGTT. INS secretion rate relative to GLU (ISR/G), and oral GLU INS sensitivity (OGIS) index were determined, using the measurements of fasting (time 0), 15, 30, 60, 90 and 120 minute plasma GLU, C-peptide and INS levels. The change from baseline to study endpoint (data censored prior to major change in INS, up to week 24) in the full analysis set was analyzed using analysis of covariance (ANCOVA) model.

**Results:** The increase in ISR/G from baseline to study endpoint in the vildagliptin group was larger than in the placebo group with statistically significant difference between treatments (Table 1). Small increases from baseline to endpoint (approximately 5%) in OGIS were observed in both treatment groups with no difference between treatment groups.

**Conclusion:** Vildagliptin added to stable dose of basal or premixed INS, with or without concomitant MET treatment, improves a chronic effect on beta-cell function. This improved beta-cell function is presumably secondary to the known diverse acute pancreatic and non-pancreatic effects of vildagliptin. Table 1: ANCOVA results for change in ISR relative to glucose (0 - 2 hours) and change in OGIS index from baseline to study endpoint by treatment.

				Difference in adjusted mean change (Vildagliptin-Placebo)		
Treatment	n	Baseline mean (SE)	Adjusted mean change (SE)	mean (SE)	(95% CI)	P-Value
ISR/G (Full-analysis set)						
Vildagliptin 50mg bid	167	8.43 (0.39)	2.92 (0.48)	2.65 (0.56)	(1.55, 3.76)	<0.001*
Placebo	144	8.21 (0.34)	0.26 (0.48)			
OGIS index (Full-analysis set)						
Vildagliptin 50mg bid	153	297.13 (9.00)	16.39 (8.19)	2.10 (9.89)	(-17.36, 21.56)	0.832
Placebo	125	304.29 (7.18)	14.29 (8.55)			

Clinical Trial Registration Number: NCT01224366

Supported by: Novartis Pharma

## 860

**Improved glycaemic control without increased hypoglycaemia incidence and weight gain with insulin-vildagliptin combination therapy in Asian patients with type 2 diabetes**P. Kozlovski<sup>1</sup>, J. Foley<sup>2</sup>, Q. Shao<sup>2</sup>, V. Lukashevich<sup>2</sup>, W. Kothny<sup>2</sup>;<sup>1</sup>Novartis Pharma AG, Basel, Switzerland, <sup>2</sup>Novartis Pharmaceutical Corporation, East Hanover, USA.

**Background and aims:** The diabetes epidemic in Asia is characterised by rapid growth, onset at a relatively young age and low BMI. Approximately 25% of patients are treated with insulin, however their glycaemic control remains unsatisfactory and the fear of hypoglycaemia remains a barrier for its improvement. Vildagliptin (VILDA) is a dipeptidyl peptidase-4 inhibitor with proven efficacy and low risk of hypoglycaemia in monotherapy and in com-

bination with oral antidiabetic drugs (OADs) and insulin (INS). Considering the specifics of the pathophysiology of type 2 diabetes (T2DM) in Asians, it is of interest to characterise the response to VILDA therapy when combined with insulin in this population.

**Materials and methods:** VILDA 50 mg bid added to INS with or without concomitant metformin therapy was studied in a 24-week, global, randomised, double-blind, placebo-controlled study. We present a post-hoc analysis of 173 Asian patients with T2DM who participated in this study (38.5% of the overall study population). Change in efficacy endpoints, HbA1c and fasting plasma glucose (FPG), from baseline to study endpoint were analysed using an analysis of covariance (ANCOVA) model. Change from baseline to endpoint in body weight was summarised by treatment. Safety and tolerability of VILDA were also evaluated.

**Results:** Patients were 54.5 years old, with T2DM duration of 11.6 years and BMI 26.4 kg/m<sup>2</sup>. They were treated with premixed INS (63.6%), long-acting INS (12.7%) and intermediate-acting INS (23.7%). The mean duration of INS use was 3.6 years and the mean dose was 39.5 U/day. Sixty percent of patients were also receiving stable metformin dose ≥1500 mg. Eighty-seven patients were receiving VILDA and 86 patients were receiving placebo. The between treatment difference in HbA1c was 0.82% (p<0.001) in favor of VILDA. There was no significant difference in the change in FPG between treatments (Table 1). The weight was stable in both treatment groups (change from baseline to endpoint 0.3 kg and -0.2 kg, for VILDA and placebo, respectively). Overall VILDA was safe and well tolerated. Incidences of hypoglycaemia were similarly low (8.0% vs 8.1%) and no severe hypoglycaemic events were experienced in either of the groups.

**Conclusion:** In Asian patients inadequately controlled with INS (with or without concomitant metformin), INS-VILDA combination treatment resulted in a significant reduction in HbA1c compared to placebo. This improvement in glycaemic control did not put patients at additional risk of hypoglycaemia or weight gain. These findings are in line with the results of the overall population in the study. INS-VILDA combination can be a suitable treatment regimen in Asian patients taking INS who do not achieve glycaemic target.

Table 1. Change in HbA1c and FPG from baseline to study endpoint (Full analysis set)

				Difference in adjusted mean change (VILDA-Placebo)		
Treatment	n	Baseline mean (SE)	Adjusted mean change (SE)	Mean (SE)	( 95% CI )	P-Value
<b>HbA1c (%)</b>						
VILDA 50mg bid	85	8.86 (0.11)	-0.80 (0.22)	-0.82 (0.10)	(-1.15, -0.50)	<0.001*
Placebo	84	9.00 (0.11)	-0.03 (0.23)			
<b>FPG (mmol/L)</b>						
VILDA 50mg bid	85	9.61 (0.35)	0.16 (0.74)	-0.53 (0.55)	(-1.62, 0.55)	0.335
Placebo	84	9.01 (0.32)	0.69 (0.76)			
FPG - Fasting Plasma Glucose; VILDA - Vildagliptin; SE - Standard Error						

FPG - Fasting Plasma Glucose; VILDA - Vildagliptin; SE - Standard Error

Clinical Trial Registration Number: NCT01224366

Supported by: Novartis Pharma

## 861

**Enhanced beta cell function after chronic treatment with the dipeptidyl peptidase-4 inhibitor Vildagliptin in an advanced age diet-induced obesity mouse model**

B. Omar, J. Vikman, M.S. Winzell, B. Åhrén;

Clinical Sciences, Lund University, Sweden.

**Background and aims:** Treatment of type 2 diabetes with dipeptidyl peptidase-4 (DPP-4) inhibitors has a potential for long-term effects on beta cell function, since DPP-4 inhibitors, as well as glucagon-like peptide-1 (GLP-1), the level of which is increased during this treatment, have been shown to increase beta-cell mass in various rodent models. However, these models have been of short duration allowing effects only during up to 10 weeks to be examined and treatment has usually commenced in young animals, which is far from the clinical situation. Therefore, more appropriate pre-clinical models are needed for the studies on long-term effects and for development of improved incretin based therapies.

**Materials and methods:** We have generated a diet induced obesity (DIO) model in advanced aged mice, to more accurately represent the time at which

hyperglycemia debuts in humans, and treated the mice with DPP-4 inhibition over a very long time. C57BL/6J mice were 10 months old at the start of high fat diet (HFD) feeding and received the high fat diet for 12 months. After one month of HFD alone, a subgroup of mice were treated with the DPP-4 inhibitor vildagliptin (3μmol/day in the drinking water), in addition to the HFD, for a period of 11 months. Control HFD mice were given normal drinking water, and the protocol also included a series of mice given normal chow throughout with or without treatment with vildagliptin. Oral glucose tolerance tests (OGTT) were administered at the start of and at 2, 5, 8 and 11 months after the start of vildagliptin treatment. At termination pancreata were excised for measurement of beta cell mass.

**Results:** Fasting glucose was reduced by vildagliptin treatment in HFD fed animals by the end of the 11 month study period ( $8.6 \pm 0.5\text{mM}$  at start v.  $6.8 \pm 0.5\text{mM}$  at 11 months after start of therapy,  $p < 0.05$ ) whereas no such reduction was observed in controls. Furthermore, HFD fed mice treated with vildagliptin had lower plasma glucose levels after an OGTT than untreated mice after two months of treatment ( $p < 0.05$ ) and glucose levels decreased continually thereafter until the end of the study period ( $p < 0.001$  at 5 and 11 months after start). Insulin secretion in response to the OGTT was increased in the HFD fed, vildagliptin treated mice after two months of treatment ( $p < 0.01$ ) and remained elevated during the 11 month study period ( $p < 0.001$  at 5 and 11 months). Beta cell function was improved by vildagliptin treatment in both diet groups at each time point throughout the course of the study ( $p < 0.001$  for all time points). In HFD fed mice, HOMA-IR was significantly lower in the vildagliptin treated group after 2 months of treatment compared to the untreated group ( $p < 0.05$ ), there were no significant differences between the groups at later time points. Despite increases in beta cell function, beta cell mass was not significantly altered by long-term vildagliptin treatment in advanced age mice in either diet group. Finally, there were no differences in weight gain between untreated and vildagliptin treated mice in either diet group.

**Conclusion:** In a unique advanced age DIO model with regular testing of islet function throughout the very old age of 21 months, insulin secretion, insulin sensitivity and beta cell function, but not mass, was improved by chronic vildagliptin treatment in HFD fed mice, and the improvements lasted over the lifetime of the experimental mice. This shows that DPP-4 inhibition has the potential of improve beta cell function over a long-term period.

Supported by: VR, Region Skåne, Lund University Medical Faculty, Novartis

## 862

**Vildagliptin more effectively achieves a composite endpoint of HbA<sub>1c</sub> <7% without hypoglycaemia or weight gain compared with SUs: a pooled analysis of clinical trials**

G. Bader, A. Schweizer;  
Novartis Pharma AG, Basel, Switzerland.

**Background and aims:** Previous studies have shown that vildagliptin as add-on therapy to metformin in patients with type 2 diabetes mellitus (T2DM) has similar efficacy on HbA<sub>1c</sub> as sulfonylureas (SUs). In this pooled analysis, we aimed to measure the overall clinical benefit of vildagliptin compared with SUs by assessing the number of patients reaching a composite endpoint of HbA<sub>1c</sub> <7%, no hypoglycaemia (symptoms and plasma glucose <3.1 mmol/L), and no weight gain (<3%). We also investigated whether there were differences in reaching the composite endpoint in relationship to age of patients and/or duration of diabetes.

**Materials and methods:** Data were pooled from two add-on to metformin studies ( $n = 3033$ ) comparing vildagliptin ( $n = 1541$ ) with SUs (glimepiride and gliclazide,  $n = 1492$ ). The relative success rate (SR) was calculated by taking the ratio of the number of patients achieving composite endpoint in vildagliptin and SU groups.

**Results:** The overall baseline HbA<sub>1c</sub> was 7.9% for both vildagliptin and SUs. After 52 weeks, a higher proportion of patients reached the composite endpoint in the vildagliptin group (32.3%) than in the SU group (21.9%). The overall SR of vildagliptin compared with SUs, along with the relative SR with respect to age of patients or duration of diabetes is presented in the table.

**Conclusion:** This pooled analysis shows that vildagliptin as add-on therapy to metformin demonstrated a better clinical benefit - as defined by the composite endpoint of reaching HbA<sub>1c</sub> <7%, with no hypoglycaemia and no weight gain - than SUs added to metformin after 52 weeks of treatment, regardless of age of patients or duration of diabetes.

Success rate (HbA <sub>1c</sub> <7%, no hypos, no weight gain)			
	Vildagliptin n/total (%)	SU n/total (%)	Vildagliptin vs. SU Relative SR (95% CI)
<b>Overall</b>	497/1541 (32.3)	327/1492 (21.9)	1.47 (1.31, 1.66)
<b>Age (years)</b>			
<50	77/299 (25.8)	43/297 (14.5)	1.78 (1.27, 2.49)
≥50 - <60	158/528 (29.9)	113/491 (23.0)	1.30 (1.06, 1.60)
≥60 - <70	206/556 (37.1)	136/541 (25.1)	1.47 (1.23, 1.77)
≥70 - <80	56/158 (35.4)	35/163 (21.5)	1.65 (1.15, 2.37)
<b>Duration of diabetes (years)</b>			
<2	94/287 (32.8)	66/267 (24.7)	1.32 (1.01, 1.73)
2 - 5	168/510 (32.9)	96/455 (21.1)	1.56 (1.26, 1.94)
>5	235/744 (31.6)	165/770 (21.4)	1.47 (1.24, 1.75)

Supported by: Novartis Pharma

## 863

**Effectiveness and safety of vildagliptin compared with other oral antidiabetic drugs in patients with type 2 diabetes: results from a large worldwide cohort study (EDGE)**

C. Mathieu<sup>1</sup>, G. Bader<sup>2</sup>, N. Hagner on behalf of EDGE Steering Committee<sup>3</sup>;

<sup>1</sup>Experimental Medicine and Endocrinology Section, Catholic University of Leuven, Belgium, <sup>2</sup>Novartis Pharma AG, Basel, Switzerland, <sup>3</sup>Novartis Pharmaceuticals Corporation, East Hanover, USA.

**Background and aims:** While metformin is an established first line treatment for patients with type 2 diabetes mellitus (T2DM), intensification of therapy with combinations is typically required over time. The purpose of the Effectiveness of Diabetes control with vildaGliptin and vildagliptin/mEtformin (EDGE) study is to compare prospectively the effectiveness and safety of vildagliptin with other oral antidiabetic drugs (OADs) in patients with T2DM inadequately controlled by monotherapy, in real life conditions across 5 regions worldwide (East Asia, Europe, Latin America, India, Middle East).

**Materials and methods:** Patients became eligible only after the add-on treatment was chosen by the physician based on the patient need and were assigned to one of two cohorts: (i) vildagliptin or (ii) other OADs including any sulfonylurea, thiazolidinedione, glinide, or α-glucosidase inhibitor or metformin but excluding any DPP-4 inhibitor or GLP-1 mimetics/analogues. The effectiveness was assessed by means of a composite endpoint including the proportion of patients responding to treatment (HbA<sub>1c</sub> drop >0.3%) without peripheral edema or proven hypoglycemic event or discontinuation due to gastrointestinal (GI) event or significant weight gain (≥5%) after 12 months of treatment.

**Results:** Overall, 45868 patients in 27 countries across the world were enrolled and assigned to a treatment cohort ( $N=29759$  in vildagliptin and  $N=16078$  in the comparator cohort), of which 31 patients were not assigned to any cohort. The mean baseline HbA<sub>1c</sub> was 8.2% in both cohorts. The probability of success [analysed using a multivariable logistic regression model to calculate odds ratios (OR) adjusted for potential confounders (baseline age, HbA<sub>1c</sub>, BMI, gender, region, co-morbidities, co-treatments)] was higher for vildagliptin, overall and in every region regardless of baseline HbA<sub>1c</sub> and second drug chosen by the physician in the comparator arm. Overall, 55.4% and 51.3% of patients in the vildagliptin and comparator cohorts, respectively, had decrease in HbA<sub>1c</sub> >0.3% without peripheral edema, hypoglycemia, GI events and weight gain after 12 months of treatment (Unadjusted OR 1.18 [95% CI: 1.13; 1.22]). Overall, 2383 (5.44%) adverse events were reported: 1503 (5.28%) in vildagliptin and 880 (5.73%) in the comparator cohort.

**Conclusions:** In real life setting, the proportion of patients responding to treatment (HbA<sub>1c</sub> drop >0.3%) without tolerability issues (hypoglycemia, weight gain, GI events and peripheral edema) was higher in the vildagliptin than in the comparator cohort in every region of the world considered, regardless of HbA<sub>1c</sub> at the baseline or drug added to the monotherapy. The proportion of reported adverse events was similar in the two cohorts.

	Vildagliptin N=28061	Comparator N=15294	Odds Ratio (95% CI)
Overall adjusted	15536/12525	7852/7442	1.49 (1.42, 1.55)
East Asia	438/1206	183/547	1.09 (0.89, 1.33)
Europe	7542/7764	3002/3457	1.12 (1.06, 1.19)
Latin America	1810/1192	391/380	1.48 (1.26, 1.73)
Middle East	1904/599	1394/870	1.98 (1.75, 2.25)
India	3842/1764	2882/2188	1.65 (1.53, 1.79)
Sulfonylureas	15536/12525	4179/3809	1.13 (1.08, 1.19)
Alpha-glucosidase inhibitors	15536/12525	357/476	1.65 (1.44, 1.90)
Thiazolidinediones	15536/12525	1206/1205	1.24 (1.14, 1.35)
Metformin	15536/12525	1835/1651	1.12 (1.04, 1.20)
Glinides	15536/12525	275/301	1.36 (1.15, 1.60)

Supported by: Novartis Pharma

## 864

### Factors contributing to the glucose-lowering effect of vildagliptin identified from the results of the OGTT in Japanese patients with type 2 diabetes

A. Nakamura, Y. Terauchi;

Department of Endocrinology and Metabolism, Graduate School of Medicine, Yokohama City University, Japan.

**Background and aims:** Several clinical trials have shown the efficacy of vildagliptin in patients with type 2 diabetes. Although OGTT is simpler, less time-consuming and less expensive to perform than the intravenous test or tracer tests, and is often used in intervention studies, the efficacy of vildagliptin has not yet been adequately examined by OGTT. Here, in order to investigate the factors contributing to the glucose-lowering effect of vildagliptin, we analyzed the results of OGTT together with several clinical parameters in Japanese patients with type 2 diabetes before and after 24 weeks of treatment with vildagliptin.

**Materials and methods:** After obtaining the approval of the institutional review board and written informed consent from the patients, we analyzed several clinical parameters, including HbA1c and body weight before and after 24 weeks of treatment with vildagliptin at 100 mg/day. Also, the plasma glucose and insulin were measured after an overnight 12-h fast and during a 75-g OGTT at 30, 60, 90 and 120 min before and after 24 weeks of treatment with vildagliptin.

**Results:** The data of the 13 patients who satisfactorily completed the follow-up examinations were included in the analysis. The mean age was  $68.9 \pm 6.7$  years. At baseline, the mean fasting plasma glucose was  $156.9 \pm 39.0$  mg/dl, HbA1c was  $7.2 \pm 0.5\%$ , and BMI was  $22.4 \pm 3.3$ . After 24 weeks treatment with vildagliptin, the patients were classified into a responder group (9/13; 69.2%) and a non-responder group (4/13; 30.8%); the responders consisting of subjects whose HbA1c decreased following 24 weeks treatment with vildagliptin, and the non-responders consisting of subjects who did not show any significant decrease of HbA1c. There were no differences in baseline characteristics between the two groups before administration of vildagliptin. After 24 weeks of treatment, HbA1c was significantly reduced from  $7.3 \pm 0.5\%$  to  $6.7 \pm 0.5\%$  in the responder group ( $P = 0.0077$ ), while it tended to rather increased from  $7.1 \pm 0.6\%$  to  $7.5 \pm 0.7\%$  in the non-responder group ( $P = 0.0679$ ). There were no differences in the degree of the body weight between the two groups. Next, we compared some parameters, including the insulin secretion and insulin sensitivity as estimated by the OGTT between the two groups before and after 24 weeks of treatment with vildagliptin. There were no differences in these parameters between the two groups before the start of administration of vildagliptin. Of note, while Matsuda index and HOMA-IR were similar in the two groups after treatment of vildagliptin, the insulinogenic index and oral disposition index were significantly higher in the responder group than in the non-responder group (insulinogenic index:  $0.27 \pm 0.22$  in the responder group versus  $0.07 \pm 0.01$  in the non-responder group ( $P = 0.0308$ ); oral disposition index:  $1.12 \pm 1.11$  in the responder group versus  $0.30 \pm 0.15$  in the non-responder group ( $P = 0.0087$ )). By contrast, the insulin/glucose ratio at 30 min and the HOMA- $\beta$  were indistinguishable between the two groups after vildagliptin treatment.

**Conclusion:** Although further large-scale randomized controlled studies are needed to obtain more detailed information, our results suggest that the difference in the degree of improvement of the glucose tolerance between the

responder group and the non-responder group was caused by the effect of vildagliptin on the insulin secretory capacity, but not on the insulin sensitivity.

## 865

### Efficacy and tolerability of vildagliptin as an add-on to nateglinide in Japanese patients with type 2 diabetes mellitus

K. Fujimaki, T. Hirose, Y. Someya, T. Yoshihara, Y. Fujitani, H. Watada; Metabolism & Endocrinology, Juntendo University Graduate School of Medicine, Bunkyo-ku, Japan.

**Background and aims:** Vildagliptin improves glycemic control in patients with type 2 diabetes mellitus (T2DM) either as monotherapy or in combination with other oral hypoglycemic drugs or insulin. In addition, vildagliptin is weight neutral and is associated with minimal risk of hypoglycemia either as monotherapy or in combination with metformin or TZD. There has been no report that shows the efficacy of vildagliptin in combination with insulin secretagogue, glinide in type 2 diabetes patients, previously. To investigate the efficacy and tolerability of vildagliptin, a potent and selective eptidyl peptidase-4 inhibitor, as add-on to nateglinide in Japanese type 2 diabetes patients controlled inadequately.

**Materials and methods:** Forty patients inadequately controlled with nateglinide (range of HbA1c was 6.9–8.4% and mean duration of use was 12 months) were randomized 1:1 to switch from nateglinide to vildagliptin (50mg twice daily, n=20: V group) or vildagliptin as add-on therapy to nateglinide (50mg of vildagliptin twice daily and 90 mg of nateglinide thrice daily, n=20: C group). Patients underwent a meal tolerance test on 90 mg nateglinide alone at weeks 0 and on 50 mg vildagliptin alone or on 50 mg vildagliptin in combination with 90 mg nateglinide at weeks 24.

**Results:** Treatment groups were balanced at baseline (glycosylated hemoglobin [HbA1c (NGSP)], 7.5%; fasting plasma glucose, 8.5mmol/L). The mean changes from baseline HbA1c to week 24 endpoint were  $-1.2 \pm 0.3$  and  $-0.3 \pm 0.5\%$  in patients of C group and V group respectively ( $P < 0.001$ ). The mean changes of glucose AUC<sub>0-180</sub> was  $-140.6 \text{ mmol} \cdot \text{min/L}$  in patients of V group compared to  $-361.2 \text{ mmol} \cdot \text{min/L}$  in those of C group ( $P < 0.001$ ). The difference between the treatment groups in HOMA- $\beta$  and HOMA-R were not significantly different. The AUCs of insulin from 0 to 180 decreased from baseline to endpoint in both groups, while there were no significant differences between the two groups (mean change  $\pm$  standard deviation [SD] =  $-518 \pm 1428 \mu\text{U} \cdot \text{min/ml}$  in patients of C group and  $-893 \pm 1808 \mu\text{U} \cdot \text{min/ml}$  in those of V group, respectively). The AUCs of insulin from 0 to 30 in the V group significantly reduced compared to the C group (mean change  $\pm$  SD =  $-35 \pm 328 \mu\text{U} \cdot \text{min/ml}$ ,  $+42.6 \pm 320 \mu\text{U} \cdot \text{min/ml}$ , respectively,  $P < 0.001$ ). Body weight at baseline was similar between the treatment groups. Body weight increased from baseline to endpoint in both groups, with greater changes observed in the C group compared to the V group (mean change  $\pm$  SD =  $+1.0 \pm 1.7$  and  $+0.4 \pm 1.6 \text{ kg}$ , respectively,  $P = 0.216$ ), but the difference was not statistically significant. There were no serious adverse events during the treatment period except three episodes of mild hypoglycemia in the C group. These symptoms were disappeared after dose reduction of nateglinide.

**Conclusion:** Vildagliptin 50mg twice-daily is effective and well tolerated as an add-on to nateglinide in Japanese patients with T2DM, with no significant weight gain and a severe hypoglycemia.

Supported by: Novartis Pharma KK



## PS 069 Screening, prevention and early management

866

### Development and evaluation of screening score for detecting undiagnosed diabetes and estimating absolute risk of future type 2 diabetes: TOPICS

Y. Heianza<sup>1,2</sup>, S. Hara<sup>2</sup>, S. Yoshizawa<sup>1</sup>, K. Kodama<sup>1,2</sup>, K. Saito<sup>1,2</sup>, S.D. Hsieh<sup>2</sup>, C. Horikawa<sup>1</sup>, H. Tsuji<sup>2</sup>, N. Yamada<sup>1</sup>, Y. Arase<sup>2</sup>, H. Sone<sup>1,2</sup>;

<sup>1</sup>Department of Endocrinology and Metabolism, University of Tsukuba Mito Medical Center, Mito, <sup>2</sup>Health Management Center, Toranomon Hospital, Tokyo, Japan.

**Background and aims:** Whether a screening questionnaire eliciting information on non-invasive clinical markers to detect undiagnosed diabetes would also accurately assess the absolute risk of future diabetes remains unknown. We developed a screening score for undiagnosed diabetes and assessed its effectiveness for both identifying the presence of diabetes and predicting future onset of diabetes.

**Materials and methods:** A total of 33335 Japanese individuals aged 18–88 y without a self-reported history of diabetes comprised the cohort for development of the screening score. We prospectively followed 7332 non-diabetic individuals who received re-examinations at 2 and 4 years after the initial assessment by the developed screening score. The absolute risk of developing diabetes was estimated according to screening scores.

**Results:** Prevalence of undiagnosed diabetes (fasting plasma glucose  $\geq 7.0$  mmol/L or HbA<sub>1c</sub>  $\geq 6.5\%$ ) was 2.9% (n=965). The diabetes score included age, sex, family history of diabetes, current smoking habit, body mass index and hypertension. At the optimal cut-off value of 8 points, sensitivity was 72.7% and specificity was 68.1%. Although only 6.4% of individuals detected by the screening (n=11024) actually had diabetes, 45.7% were in a pre-diabetic state. Results of Kaplan-Meier analysis demonstrated that the absolute risk for the development of diabetes gradually escalated with increases in scores (log-rank testing,  $p < 0.001$ ). Screening test results of  $< 4$ , 4–5, 6–7, 8–9 and  $\geq 10$  points at the initial examination estimated 4-y cumulative probabilities of 0.1%, 0.9%, 2.3%, 4.2% and 8.4%, respectively, for the development of future diabetes. A substantially increased risk of developing diabetes was observed for a score of 8–9 points (incidence rate, 12.6/1000 person-years) and  $\geq 10$  points (23.3/1000 person-years) than that for lower scores or that for the total subject population. Hazard ratios (HRs) for the development of diabetes were significantly higher for individuals with scores of 8–9 points (HR 1.83, 95% CI: 1.32–2.52) and scores of  $\geq 10$  points (HR 3.48, 95% CI: 2.49–4.88) compared to those with 6–7 point scores.

**Conclusion:** Prognostic information on the risk of developing diabetes could also be provided at initial screening for undiagnosed diabetes. Our study results will assist lay people in measuring their own risk of diabetes outside of clinical settings as well as targeting individuals who need to be followed to prevent new onset of the disease.

*Supported by: The Ministry of Health, Labour and Welfare, Japan*

867

### Incidence of diabetes is reduced by low-intensity, community-based lifestyle intervention. Three-year results from the Greek arm of the DEPLAN study

A. Tsiakou, S. Liatis, C. Stathi, D. Perrea, S. Grammatikou, N. Katsilambros, K. Makrilakis;

First Department of Internal Medicine & Diabetes Center, Athens University Medical School Laiko Hospital, Greece.

**Background and aims:** It has been repeatedly shown that type 2 diabetes can be prevented or delayed by intensive lifestyle intervention in populations with impaired glucose tolerance. It is not clear, however, whether less structured, community-based interventions in individuals at high type 2 diabetes risk, are effective in preventing the disease, especially in the long-term. In the present analysis, three-year incidence of type 2 diabetes was examined in a group of high-risk individuals who participated in the Greek arm of a European type 2 diabetes prevention study (DEPLAN).

**Materials and methods:** High-risk individuals, identified by the FINDRISK score questionnaire, participated in a one-year lifestyle intervention programme, based on bimonthly, group (6–8 persons) sessions with a dietitian.

All sessions took place near the participants' residence or work-place. Glycaemic status was assessed at baseline and after three years (two years after the end of the intervention) by an OGTT. A control group comprised individuals who were also screened by OGTT at baseline and followed-up for three years, who were at lower risk or did not wish to participate in the intervention programme.

**Results:** Ninety-one persons (51 females, mean age: 54.6 [52.4–56.7] years), were included in the intervention group, while the control group comprised 168 individuals (81 females, mean age: 54.2 [52.6–55.8] years). The two groups had different baseline characteristics, the intervention group being at significantly higher risk than the controls (mean FINDRISK score: 17.4 [16.9–17.9] vs. 10.3 [9.7–10.9],  $p < 0.001$  and mean fasting plasma glucose (FPG): 103.8 [101.6–106.1] mg/dl vs. 96.9 [95.2–98.5] mg/dl,  $p < 0.001$ ). At three years, individuals in the intervention group showed a 1.22% [0.2–2.6] weight loss, vs. a 1.20% [0.4–2.1] increase in the control group ( $p = 0.003$ ). In addition, a significantly higher proportion of individuals in the intervention group lost  $> 5\%$  of their initial body weight (23.1% vs. 11.4%,  $p = 0.01$ ). Incidence of diabetes at three years was similar between the two groups: 11.0% in the intervention vs. 10.1% in the control group (OR: 1.1 [0.5–2.5],  $p = 0.82$ ). When adjusted, however, for baseline FINDRISK score and baseline FPG, the intervention turned out to be a significant protective factor for incident diabetes (OR: 0.15 [0.05–0.49],  $p = 0.002$ ).

**Conclusion:** A low-intensity, community-based lifestyle intervention of one-year's duration is protecting from incident diabetes after three years. A mild weight loss is still apparent two years after the end of the intervention.

*Supported by: EU*

868

### Physical activity in screen-detected patients with abdominal obesity and metabolic syndrome: at screening and three years thereafter

H. Jansen, C. den Engelsens, G.E.H. Rutten;

Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Netherlands.

**Background and aims:** Metabolic syndrome (MetS) is associated with an increased risk of developing type 2 diabetes and cardiovascular disease. An important way to prevent this is to decrease weight and increasing the level of physical activity (PA). Our aim was to investigate the change in level of PA in patients with screen-detected metabolic syndrome.

**Material and methods:** After a population-based screening, 1,721 patients with a self-measured increased waist circumference underwent a physical examination and laboratory assessment. Totally 473 were diagnosed with MetS (NCEP ATP III criteria) and 1,248 with abdominal obesity but without MetS. All screening participants were given the advice to contact their general practitioner. Patients with MetS received 'standard' care according to existing guidelines; deliberately no special intervention was developed. Three years after screening a random selection was invited for follow-up measurements. PA was measured by means of the validated SQUASH questionnaire. Primary outcome measure: % of people fulfilling Dutch Physical Activity Guideline (DPAG) criterion.

**Results:** Mean (SD) age of our study population was 48.3 (10.9) years. At screening 55.2% of the 473 patients with MetS fulfilled the DPAG criterion compared to 61.2% of the patients 1,248 without MetS ( $p = 0.03$ ). The percentage of patients fulfilling the DPAG criterion did not increase between screening and follow-up, neither in MetS patients (n=191; 52.4% vs. 58.5%,  $p = 0.16$ ) nor in patients without MetS (n=173; 63.4% vs. 63.6%,  $p = 1.00$ ). Mean (SD) waist circumference decreased in MetS patients who increased their level of PA (n=31; 102.6(8.6) vs. 100.1(10.1),  $p = 0.03$ ) as well as in those who did not (n=160; 105.5(9.6) vs. 101.7(11.4),  $p < 0.001$ ). Eleven patients started with glucose-lowering medication between screening and follow-up. Excluding these patients, mean (SD) fasting glucose increased in MetS patients who increased their level of PA (n=26; 5.0(0.6) vs. 5.2(0.6),  $p = 0.005$ ), as well as in those who did not (n=155; 5.1(0.6) vs. 5.4(0.7),  $p < 0.001$ ).

**Conclusion:** Screen-detected patients with MetS are less physically active compared to patients without MetS. Most MetS patients do not increase their level of PA after being diagnosed with MetS and receiving usual care in a primary care setting. More effort is needed to activate these patients to prevent future diabetes.

*Supported by: Merck Sharp & Dohme*

## 869

**Genetic variation in the fat mass and obesity-associated gene (FTO) could influence food preference in adults**L. Brunkwall<sup>1</sup>, U. Ericson<sup>1</sup>, S. Hellstrand<sup>1</sup>, B. Gullberg<sup>2</sup>, M. Orho-Melander<sup>1</sup>, E. Sonestedt<sup>1</sup>;<sup>1</sup>Clinical Sciences, Diabetes and Cardiovascular Disease, Genetic Epidemiology, <sup>2</sup>Clinical Sciences, Nutritional Epidemiology, Malmö, Sweden.

**Background and aims:** Studies have indicated that the FTO genotype is not only associated with BMI and weight but also with dietary intake. Our aim was to investigate if FTO genotype (rs9939609) associates with food preferences. Additionally we examined the association separately in lean and overweight individual's challenging the question if the associations could be modified by obesity status.

**Materials and methods:** Analyses were performed among 22,799 adults from the population based Malmö Diet and Cancer Study cohort. All individuals had complete dietary data, information about the FTO genotype (rs9939609) and no history of cancer, diabetes or CVD. For analyses of food preferences we used 27 food groups estimated with a 168-item questionnaire and a 7-day menu book.

**Results:** After correcting for multiple testing we observed a significant difference for reported intake of cakes and soft drinks across the FTO genotypes (P for trend <0.0001 for both) where the A-allele carriers that are more susceptible for obesity reported lower consumption of soft drinks but higher consumption of cakes than the TT carriers. Nominal differences were seen for fruits, cereals, high fat meat, ice cream and cheese where the risk allele carriers indicated a higher consumption. These observations remained similar after excluding the potentially inadequate energy reporters. In contrast to our hypothesis the results did not differ depending on obesity status.

**Conclusion:** We observed an association between the variation in the FTO genotype and food preferences in healthy adults.

*Supported by: The Swedish Medical Research Council, Skåne University Hospital*

## 870

**Prevention of type 2 diabetes with Mediterranean diet. Preliminary results from the first year of intervention of the Egabro-Pizarra study**S. Valdés<sup>1</sup>, M. Corpas<sup>2</sup>, M. Leña<sup>1</sup>, E. Rubio-Martín<sup>1</sup>, S. Morcillo<sup>1</sup>, F. Lima-Rubio<sup>1</sup>, A. Chicano<sup>1</sup>, G. Martín<sup>1</sup>, N. Porras<sup>1</sup>, J. Gómez-Zumaquero<sup>1</sup>, F. Sorriquer<sup>1</sup>, G. Rojo-Martínez<sup>1</sup>;<sup>1</sup>Endocrinology and Nutrition, Hospital Universitario Carlos Haya, IBIMA, CIBERDEM, Málaga, <sup>2</sup>Endocrinology and Nutrition, Hospital Universitario Reina Sofia, Cordoba, Spain.

**Background and aims:** Type 2 diabetes (DM2) represents one of the greatest health problems in our time. Its increasing proportions are especially worrisome in the south of Spain. The only alternative to stop the increasing incidence is prevention. The aim of this study is to test if an intervention program based on changes in lifestyle according to the Mediterranean diet pattern may: 1- prevent the incidence of type 2 diabetes in at least 50% and 2- reverse newly diagnosed diabetes in a semi-urban population of Andalusia (South of Spain). Another objective is to detect which factors influence the success of the intervention.

**Materials and methods:** Population-based cohort study including 320 subjects selected from the general population of two semi-urban towns of the south of Spain (Cabra and Pizarra) after performing an OGTT showing some glucose disturbance: Impaired fasting glucose (IFG) and / or impaired glucose tolerance (IGT) or undiagnosed diabetes. Individuals in the Cabra cohort (n=160) were included in an intensive lifestyle program with regular visits to achieve goals for dietary habits, exercise and weight within the Mediterranean dietary pattern (Intervention group). Individuals in the Pizarra cohort (n=160) were referred to their usual physicians with standard recommendations on lifestyle changes (Control group).

**Results:** After one year follow-up, diabetes developed in 9.1% of individuals with IFG and / or IGT in the control group compared to 4.7% in the intervention group (p<0.01). In this group, 60% had a complete reversion of glucose disturbance (compared to 47% in the control group). Of those with newly diagnosed diabetes, 46.7% reverted to either IFG/IGT or normal in the control group versus 73% in the intervention group (p<0.01). The intervention had strong effects on glucose and anthropometry measurements. No baseline anthropometric variable predicted the effect of treatment or the evolution of the subject within the year but Improving blood glucose was associated with weight loss during monitoring period.

**Conclusion:** After one year of intervention, we have shown that hyperglycemia (even at diabetic level) can be normalized without medication through changes in lifestyle following the Mediterranean diet pattern.

*Supported by: Grants from the Consejería de Salud Junta de Andalucía, ISCIII, CIBERDEM*

## 871

**Influence of working status on diabetes control - data from the PROGENS study**E. Franek<sup>1</sup>, A. Milczarczyk<sup>1</sup>, B. Mospan<sup>2</sup>, A. Sienkiewicz<sup>3</sup>, A. Zonenberg<sup>4</sup>, P. Bijos<sup>5</sup>, on behalf on PROGENS Study investigators;<sup>1</sup>Dept of Internal Diseases, Endocrinology and Diabetology, Central Clinical Hospital MSWiA, Warsaw, <sup>2</sup>Diabetology and Diabetic Foot Center, Zielona Gora, <sup>3</sup>Diabetes Center, Kolobrzeg, <sup>4</sup>Diabetes Center, Białystok, <sup>5</sup>Bioton SA, Warsaw, Poland.

**Background and aims:** Work may change the daily life habits, like eating time and volume. The aim of the present study was to answer the question whether those changes are significant and do influence diabetes control.

**Materials and methods:** To answer this question a subanalysis of data from the PROGENS study (multicenter observational study assessing effectiveness and safety of recombinant human insulin manufactured by Bioton, Poland) was performed. In this subanalysis 866 working (338F, 528M, W-group) and 1089 non-working patients with type 2 diabetes (743F, 346M, NW-group) were included (mean age 56±7 vs. 69±7y, p<0.001, mean BMI 30.3±4.8 vs. 30.8±4.7, NS, respectively). Four-points glycemic profiles, HbA1c and insulin doses were compared between those 2 groups. In a subset of 425 patients it was also compared whether there are differences in meal time and volume as well as fasting and postprandial glycemia between working and non-working patients on working and free days.

**Results:** The average daily insulin dose was similar in the both groups at study start (W 0.43±0.24 vs NW 0.45±0.23 U/kg/day, NS) as well as at the end (W 0.48±0.25 vs. NW 0.49±0.24 U/kg/day, NS). HbA1c decreased significantly during the study period (6 months) in both groups (W 8.3%±1.4 vs. 7.4%±0.9, p<0.001, NW 8.1%±1.1 vs. 7.3%±0.8, p<0.001). Similarly, significant decrease of fasting (W 169±33 vs. 140±20 mg/dl, p<0.001, NW 167±31 vs. 142±23 mg/dl, p<0.001). No significant differences in the volume and time of meals were observed, as well as no clinically meaningful differences of fasting and postprandial glucose concentrations. Working patients however eat their breakfast on average half an hour earlier (7.37±0.52 vs. 08.01±0.47, p<0.001), and their dinners have more often greater volume on working days as compared with free days (phi correlation 0.529, p<0.001). These differences however did not influence glycemic control. There were also no differences in hypoglycemia rate between working and non-working patients on working and free days. The frequency of hypoglycemia was however very small.

**Conclusion:** The differences in volume and time of meals caused by daily working schedule are slight and did not influence diabetes control in a clinically meaningful way.

*Supported by: BIOTON S.A., Warsaw, Poland*

## 872

**SLC47A1 gene rs2289669 G>A variant influences plasma lactate concentrations in metformin-treated type 2 diabetes mellitus**F. Liu<sup>1,2</sup>, Y. Shen<sup>1</sup>, T. Zheng<sup>1,3</sup>, J. Tang<sup>1,3</sup>, W. Jia<sup>1,4</sup>;<sup>1</sup>Endocrinology and Metabolism, Shanghai Clinical Medical Center of Diabetes, <sup>2</sup>Shanghai Jiaotong University-affiliated Sixth People's Hospital, <sup>3</sup>Shanghai Key Laboratory of Diabetes, <sup>4</sup>Shanghai Jiaotong University Affiliated Sixth People's Hospital, China.

**Background and aims:** The SLC47A1 gene encodes for multidrug and toxin extrusion 1 (MATE1) which plays a pivotal role in the metabolism and excretion of metformin. The present study is to investigate the relationship between the SLC47A1 gene polymorphism and plasma lactate levels in Chinese Hans who suffered from type 2 diabetes mellitus (T2DM) with and without metformin therapy.

**Materials and methods:** The SLC47A1 single nucleotide polymorphism (SNP) rs2289669 G/A was genotyped in 287 T2DM patients, including a metformin treated group (n = 151) and a non-metformin-treated group (n = 136) with PCR-restriction fragment length polymorphism method. Fasting plasma lactic acid levels were measured with an enzyme electrode assay.

**Results:** Three SLC47A1 genotypes, GA, GG and AA were found in T2DM patients of Chinese Hans. The frequency of the SLC47A1 rs2289669 G/A and A allele was 50.6%. The metformin group had higher plasma lactate concentration than the non-metformin group ( $1.15 \pm 0.36 \text{ mmol/L}$  vs  $1.01 \pm 0.37 \text{ mmol/L}$ ,  $P=0.002$ ). Patients with the mutant genotype (AA) had a higher blood lactate concentration in the metformin group than those in the non-metformin group ( $1.26 \pm 0.40 \text{ mmol/L}$  vs  $0.94 \pm 0.27 \text{ mmol/L}$ ,  $P=0.001$ ), whereas there were no obvious differences of lactate levels in patients with GA, GG genotype between the two groups. Female patients with the AA genotype had higher lactic acid concentrations ( $1.26 \pm 0.35 \text{ mmol/L}$ ) than males ( $0.99 \pm 0.36 \text{ mmol/L}$ ) with AA genotype ( $P=0.008$ ). However, there were no gender difference of lactate levels in patients with GA, GG genotype ( $P=0.061$ ,  $P=0.236$ , respectively) in total patients. In the metformin-treated group, there were significant gender differences in lactate concentrations carried with AA genotype ( $1.43 \pm 0.33$  in women vs  $1.12 \pm 0.41 \text{ mmol/L}$  in men,  $P=0.041$ ) but not with GG, GA genotype. The lactate levels of women with the AA genotype were the highest ( $1.43 \pm 0.33$  vs  $1.19 \pm 0.32$  and  $1.09 \pm 0.24 \text{ mmol/L}$ ,  $P=0.021$ ). But differences in lactate levels among the three genotypes were not observed in non-metformin group ( $P>0.05$ ).

**Conclusion:** The SLC47A1 gene rs2289669 G>A variant influences the plasma lactate concentrations in T2DM patients with metformin therapy. Lactic acid levels of female patients with AA genotype are more likely to elevate.

Supported by: National Science Foundation Items of China (81070650)

## 873

### Increased lean body mass significantly improves bone health in type 2 diabetes mellitus

I. Maisnam, D. Dutta, A. Shrivastav, S. Ghosh, S. Mukhopadhyay, S. Chowdhury;  
Endocrinology and Metabolism, Institute of Post-graduate Medical Education and Research, Kolkata, India.

**Background and aims:** Bone mass in type 2 diabetes may be low, high or similar compared to general population. High BMI and hyperinsulinemia may increase bone mass. Adiposity seen in type 2 diabetes is associated with increase cytokines, vitamin D deficiency and may affect bone health. Lean body mass may bear a positive correlation with bone health, and fat mass may have a negative correlation. The aim was to determine the correlation between body mass, body mass composition, fat distribution and bone mineral content and density; and vitamin D levels in type 2 diabetes.

**Materials and methods:** This was a cross-section study of type 2 diabetes, age 35–55 years from our diabetic clinic from December 2011 to December 2012. Post-menopausal women, prolonged immobilization, advanced kidney and liver disease, cancers, rheumatologic conditions, pregnancy, prolonged corticosteroid use were excluded. BMI  $\geq 25$  was obese based on the Indian criteria. DEXA measured bone mineral density (BMD) ( $\text{g/cm}^2$ ), bone mineral content (BMC), fat mass, lean mass, and percent body fat, android and gynoid fat with a Lunar DPX NT densitometer. 25(OH) vitamin D level was measured by chemiluminescent microparticle assay. Levels  $< 20$ ,  $20$ – $30$ ,  $> 30 \text{ ng/ml}$  were deficient, insufficient and sufficient respectively. Statistical analysis was done using SPSSv16 software. Comparison was done using one way ANOVA. Pearson's correlation coefficient was used to compare 2 variables. A  $p$  value  $< 0.05$  was considered significant.

**Results:** There were 40 females and 48 males, mean age  $40.7 \pm 5.1$  and  $47.4 \pm 5.8$  respectively. 30, 40 and 18 patients were vitamin D deficient, insufficient and sufficient respectively. A significant ( $p=0.009$ ) correlation between the BMC and the BMI ( $r=0.457$ ) was seen. A very significant ( $p<0.001$ ) strong correlation between bone mineral content and lean mass ( $r=0.847$ ) was seen, this persisted after adjusting for vitamin D levels ( $r=0.85$ ,  $p<0.001$ ). A significant fair correlation existed between BMC and fat mass ( $r=0.41$ ,  $p=0.019$ ). Lumbar spine BMD had a significant correlation with lean and fat mass ( $r=0.42$ ). Fat percent was higher, lean mass lower, BMC, BMD was lower in vitamin D deficient, but not significant. Calcium was significantly lower in the deficient group. No significance difference in HbA1c, LDL and HDL in 3 vitamin D groups. Favourable findings were seen in vitamin D sufficient and the insufficient, but not deficient groups.

**Conclusion:** Increased BMC and BMD were associated with increased lean body mass, fat mass also contributed. The association persisted after adjusting for vitamin D levels. Though insignificant, vitamin D was lower with higher fat mass and lower lean mass. Higher vitamin D was associated with higher BMD in the femur (almost significant). There was no relation between vitamin D status and glycemia. Increase lean mass is needed for good bone health

in type 2 diabetes. Adipose tissue mass which is associated with lower vitamin D levels should be decreased.

Vitamin D status, body fat composition, bone mineral content, dyslipidaemia and glycaemia

Parameter	vitamin D <20ng/ml	vitamin D 20–30ng/ml	vitamin D >30ng/ml	p-value
HbA1c	8.3 $\pm$ 2.6	7.1 $\pm$ 1.5	7.45 $\pm$ 1	0.515
BMI (kg/m <sup>2</sup> )	24.71 $\pm$ 3	25.5 $\pm$ 3.22	24.6 $\pm$ 5	0.782
Body fat %	37.9 $\pm$ 8.3	34.1 $\pm$ 6.2	31.3 $\pm$ 9.9	0.612
Lean mass g	35448.8 $\pm$ 57734	40315 $\pm$ 6365	38232 $\pm$ 6675	0.288
Gynoid fat %	41.8 $\pm$ 10.2	35.6 $\pm$ 8	34.8 $\pm$ 8.6	0.238
Android fat %	46.9 $\pm$ 6.3	45.4 $\pm$ 4.8	40.03 $\pm$ 1.09	0.154
BMD (Right femur) g/cm <sup>2</sup>	0.9481 $\pm$ 0.17	1.1276 $\pm$ 0.18	1.0171 $\pm$ 0.09	0.056
BMC g	2215 $\pm$ 3.09	2587 $\pm$ 4.7	2428 $\pm$ 4.3	0.211
Calcium mg/dl	8.9 $\pm$ 0.45	9.3 $\pm$ 0.4	9.6 $\pm$ 3.6	0.008
LDL mg/dl	126 $\pm$ 4.14	126 $\pm$ 2.6	97 $\pm$ 9.8	0.683
HDL (mg/dl)	43.5 $\pm$ 1.4	43.5 $\pm$ 7.9	44.3 $\pm$	0.856

Supported by: RSSDI, West Bengal, India

## 874

### The association between hypovitaminosis D and metabolic syndrome is not dependent on body fat mass

I. Barchetta<sup>1</sup>, D. Capoccia<sup>2</sup>, M.G. Baroni<sup>3</sup>, M. De Bernardinis<sup>1</sup>, C. Costantino<sup>1</sup>, F. Leonetti<sup>2</sup>, M.G. Cavallo<sup>1</sup>;

<sup>1</sup>Dept. Internal Medicine and Medical Specialties, Sapienza University of Rome, <sup>2</sup>Dept. of Medical Pathophysiology, Sapienza University of Rome, <sup>3</sup>Endocrinology and Metabolism, Department of Medical Sciences, University of Cagliari, Italy.

**Background and aims:** Metabolic syndrome (MS) and hypovitaminosis D represent two of the most diffuse conditions worldwide, reaching pandemic proportions in industrialized countries, and are both tightly associated with obesity. Aim of this study was to determine if an independent association exists between low 25(OH)vitamin D3 levels and MS in obese patients.

**Materials and methods:** For this purpose we recruited 107 consecutive obese subjects, 61 with MS (age (mean $\pm$ SD):  $45.3 \pm 13.3$ , BMI:  $43.1 \pm 8.3 \text{ kg/m}^2$  and 46 without MS (age:  $41.8 \pm 11.5$ ,  $p=\text{n.s.}$ , BMI:  $41.6 \pm 6.5 \text{ kg/m}^2$ ,  $p=\text{n.s.}$ ) comparable for sex, BMI, waist circumference and body fat mass, evaluated by bioimpedanzimetry. All participants underwent a complete workup including physical examination, blood testing and serum 25(OH)vitamin D3 measurement. Insulin resistance was estimated by HOMA-IR, ISI and QUICKI indexes.

**Results:** Serum 25(OH)vitamin D3 levels were significantly reduced in obese patients with MS compared with obese subjects without MS ( $14.9 \pm 6.2 \text{ ng/ml}$  vs  $18.9 \pm 8.2 \text{ ng/ml}$ ,  $p<0.007$ ). The multivariate regression analysis confirmed that low serum 25(OH)vitamin D3 levels were associated with the diagnosis of MS in obese patients independently from gender, age serum PTH and body fat mass. After stratifying the study population according to 25(OH)vitamin D3 levels, patients in the lowest vitamin D quartile showed a markedly increased prevalence of MS with an OR of 4.1 (CI 1.2–13.7,  $P=0.02$ ) compared to those in the highest quartile.

**Conclusion:** A powerful association exists between hypovitaminosis D and MS in obese patients independently from body fat mass and its clinical correlates. This indicates that the association between low serum 25(OH)vitamin D3 levels and MS is not merely induced by vitamin D deposition in fat tissue and reinforce the hypothesis that hypovitaminosis D represent a crucial independent determinant of MS.

Supported by: MIUR (PRIN grant 2008) and Sapienza University of Rome



## 875

**Effect of Ramadan fasting on diabetes control in type 2 diabetics**

H. Ibrahim, H. Ouertani, A. Dorai, A. Jaidane, C. Zouaoui, B. Zidi;  
Service of Endocrinology Diabetology, Military Hospital – Monfleury,  
Tunis, Tunisia.

**Background and aims:** To determine effects of the intervention of education and study of Ramadan fasting on the metabolic control diabetes.

**Materials and methods:** 100 diabetic patients were recruited two months before the start of Ramadan. Pre-Ramadan counseling, and education was given to all patients on diet, drug and acute complications of diabetes. People with complication fluctuating blood sugar and no self monitoring blood sugar were excluded from trial. Following parameters were checked before and one month of completing 30 days of Ramadan Fasting (age, sex, weight, treatment, FBS, Lipid profile, number of meals, physical activity, HbA<sub>1c</sub> and Hypoglycaemia episodes during Fasting). 87 were on OHA, 9 on insulin and 4 on combination therapy.

**Results:** 37 patients lost weight, 51 gained weight, no weight change was seen in 12. HbA<sub>1c</sub> decreased in 58, increased in 38, did not change in 4. Patients who took 3 meals as compared to 2 showed increase in FBS (126 mg/dl to 138), HbA<sub>1c</sub> (7,2 % to 7,8) and cholesterol (188 mg/dl to 210). While patients who had 2 meals showed improvement in HbA<sub>1c</sub> (7,6 % to 6,8), cholesterol (188 mg/dl to 176). Although compared to pre-Ramadan, the post Ramadan data showed increase in FBS (128 to 135 mg/dl) but avg. HbA<sub>1c</sub> reduced from 8,3% to 7,5%. Cholesterol from 188 to 178 mg /dl suggesting good effect of Ramadan fasting on HbA<sub>1c</sub> and lipid profile. During Ramadan month 13 episodes of hypoglycaemia but only 2 were severe enough to break the fast but no requiring hospital admission. 11 episodes were in OHA group only 2 in insulin taking patients.

**Conclusion:** Ramadan fasting in diabetics can be safe, but it needs more education in pre-Ramadan counselling of both patients and their family members especially in terms of diet and drug compliance.

## PS 070 Nutrition, diet and diabetes

## 876

**Hepatic fat content is related to the carbohydrate intake, in patients with non alcoholic fatty liver disease (NAFLD)**

V. Rigalleau, C. Gonzalez, J. Vergniol, J. Foucher, F. Chermak, S. Carlier,  
E. Maury, B. Cherifi, H. Gin, V. de Ledinghen;  
USN, Hopital Haut-Lévêque, Pessac, France.

**Background and aims:** NAFLD are now the first cause of hepatic diseases, and their first treatment is diet. According to studies by magnetic resonance, the drastic reduction of the carbohydrate intake (20-30 grams/day) during two weeks leads to a half-reduced hepatic fat content. Is hepatic steatosis related to the spontaneous carbohydrate intake in patients with NAFLD?

**Materials and methods:** We performed dietary records (BillNutIV software) in 24 patients with NAFLD, 3±2 months after their hepatic biopsy was performed, and before the deliverance of a dietary advice. Other causes of liver diseases (virus, drugs) were excluded, as were the subjects who declared an excessive alcohol consumption (>20g/d for women and 30g/d for men), and the subjects who had known diabetes, that might have led them to restrain their carbohydrate intake. The food quotient, indicator of the proportion of calories from carbohydrates, was calculated as: (1.00X % calories from carbohydrates/100) +(0.70X % calories from lipids/100) +(0.81 X % calories from proteins/100). The associations between diet variables and % steatosis on the hepatic biopsies were tested by regression analysis, and diet variables were compared according to the presence of fibrosis and inflammation on the biopsies.

**Results:** The subjects (15 men, 9 women, age 45±13 yrs, BMI 29.7±3.8, ALAT 72±53U, GGT 117±98U) displayed a large range of hepatic steatosis: 50.5%±25.5 [10-90], correlated to their energy intake (1993±597 kcal/d, r=0.41, p<0.05) and their food quotient (0.85±0.02, r=0.42, p<0.05), which remained significant with both variables by a multivariate regression analysis (r=0.51, p<0.05). For the 17/24 patients with a hepatic fibrosis, the energy intake was lower (Fibrosis:1863±503 vs others: 2382±733 kcal/d, p<0.05), and the history of previous overweight was more pronounced as reflected by a higher maximal BMI during their life (Fibrosis: 33.2±3.4 vs others: 27.4±4.0 kg/m<sup>2</sup>, p<0.05). For the 15/24 patients with hepatic inflammation on the biopsy, we did not detect any difference in the diet.

**Conclusion:** Hepatic steatosis is related to the energy and carbohydrate intakes in our patients who had a hepatic biopsy for NAFLD: the role of dietary carbohydrates is detectable in the range of usual carbohydrate intake (32% to 58% of calories were from carbohydrates in our patients). The carbohydrate proportion did not differ in the patients with hepatic fibrosis.

## 877

**Effects of the prudent diet versus low fat diet in cytokines profile in patients with diabetes and chronic hepatitis C**

E.D. Rusu<sup>1</sup>, M. Jinga<sup>2</sup>, G. Enache<sup>3</sup>, F. Rusu<sup>4</sup>, A. Dragomir<sup>5</sup>, I. Ancuta<sup>2</sup>,  
R. Dragut<sup>5</sup>, R. Nan<sup>5</sup>, I. Sima<sup>6</sup>, S. Ateia<sup>7</sup>, V. Stoica<sup>8</sup>, D. Cheta<sup>9</sup>, G. Radulian<sup>5</sup>;

<sup>1</sup>Nutritioiabetes, D, University of Medicine and Pharmacy, Bucharest,

<sup>2</sup>Gastroenterology, University of Medicine and Pharmacy, Bucharest,

<sup>3</sup>Diabetes, Emergency Hospital, Contanta, Romania, <sup>4</sup>Urology, Emergency Military Hospital Carol Davila, Bucharest, <sup>5</sup>Diabetes, University of Medicine and Pharmacy, Bucharest, <sup>6</sup>Diabetes, National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, <sup>7</sup>Diabetes, Medas Clinic, Bucharest,

<sup>8</sup>Gastroenterology, University of Medicine Carol Davila, Bucharest, Romania.

**Background and aims:** Chronic hepatitis C (CHC) is characterized by a chronic inflammatory status in the liver, with increased production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), which may enhance the IR. The aim of this study was to compare the prudent diet (PD) with a low-fat diet (LFD) in patients with CHC and diabetes. The purpose was to measure the impact of dietary changes on reduction of insulin resistance, and on improving the cytokines profile (adiponectin, leptin, TNF-α, IL-6, resistin).

**Materials and methods:** This multicenter, randomized controlled trial was conducted during September 2007 - December 2010. 120 participants were recruited from three hospitals from Bucharest, Romania. The inclusion criteria were: age over 35 years, diagnosis of chronic hepatitis C (CHC infection was defined by the presence of anti-HVC for a least 6 months and a positive

HCV-viremia) and diabetes. Following completion of baseline assessments, participants were randomized to the prudent diet (PD), or to the low fat diet (LFD) group, both with a lifestyle management program. We performed anthropometric measurements (weight, height, BMI (body mass index), waist circumference, waist to hip ratio (WHR) every month. The biochemical analyses, which included fasting serum lipids, glucose profile, liver profile, were performed at baseline, 6 and 12 months. The serum concentrations of adiponectin, leptin, resistin, TNF- $\alpha$ , IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) at baseline and 12-months.

**Results:** After 6 and 12 months of intervention, both groups had a significant decrease in caloric consumption. At 6 months, weight loss was greater in the PD group ( $-5.02 \pm 3.43$  kg vs.  $-4.1 \pm 2.6$  kg;  $p=0.002$ ) compared to the LFD group. At 1-year, however, weight loss was similar in both groups ( $-3.9 \pm 3.3$  kg vs.  $-3.1 \pm 2.6$  kg;  $p=0.139$ ). HOMA-IR was normalized in 10.3% of PD patients ( $n=3$ ) and 4% ( $n=1$ ) of patients in LFD and improved to 46.2% ( $n=6$ ) of PD and 15.4% ( $n=2$ ) of the patients in the LFD. With both diet leptin, TNF- $\alpha$ , IL-6 and resistin decreased and adiponectin increased with significant differences; also there were significant improvements in AST/ALT ratio, AST/Platelets ratio and Forns index.

**Conclusion:** Patients with hepatitis C following a lifestyle intervention for 1-year had significant improvements in overweight or obesity, fasting glucose, fasting insulin, HOMA-IR, lipid, hepatic and cytokine profile with both diets. Supported by: PNCDI 2 program DIADIPOHEP 41-008/2007

## 878

### Effects of dietary polyphenols and/or n-3 fatty acids on postprandial lipaemia in people at high cardiovascular risk: the Etherpaths project

G. Annuzzi<sup>1</sup>, L. Bozzetto<sup>1</sup>, G. Costabile<sup>1</sup>, G. Della Corte<sup>1</sup>, G. Anniballi<sup>1</sup>, M. Vitale<sup>1</sup>, S. Cocozza<sup>1</sup>, R. Giacco<sup>2</sup>, C. Vetrani<sup>1</sup>, A. Rivieccio<sup>1</sup>, L. Patti<sup>1</sup>, G. Della Pepa<sup>1</sup>, G. Riccardi<sup>1</sup>, A.A. Rivellese<sup>1</sup>

<sup>1</sup>Department of Clinical and Experimental Medicine, Federico II University,

<sup>2</sup>National Council Research, Naples, Italy.

**Background and aims:** Postprandial lipaemia is an independent risk factor for cardiovascular disease. Among the dietary elements potentially able to reduce the cardiovascular risk much interest is currently placed on polyphenols and n-3 fatty acids. Their effects on postprandial lipid metabolism, especially when combined, are not known. Therefore, the aims of this study were to evaluate the effects of diets rich in polyphenols and/or n-3 fatty acids and their possible interaction on triglyceride-rich lipoprotein levels after a fat test meal in people at high cardiovascular risk.

**Materials and methods:** Seventy-four individuals, age 35–70 years, with high BMI ( $27\text{--}35$  kg/m<sup>2</sup>) and waist circumference (men  $>102$  cm, women  $>88$  cm), and one more component of the metabolic syndrome, were randomized to one of the following diets for 8 weeks: (A) poor in n-3 fatty acids and polyphenols, (B) rich in n-3 fatty acids, (C) rich in polyphenols, (D) rich in n-3 fatty acids and polyphenols. The diets were isocaloric and similar for content of MUFA, saturated fat, cholesterol, carbohydrates, fibre, proteins, and vitamins. Before and after the 8-week intervention, lipid concentrations in plasma and lipoprotein subfractions separated by density gradient ultracentrifugation were determined, both at fasting and over 6 hours after a high fat meal of the same composition as the diet assigned.

**Results:** The results refer to the 63 participants who have already completed the study, 19 for diet A, 14 for diet B, 15 for diet C, and 15 for diet D. Adherence to diet was optimal in all groups. The postprandial incremental areas under the curve (iAUC) of cholesterol and triglycerides in chylomicrons were reduced with diets B, C, and D with respect to the control diet A, significantly for cholesterol between diet A and B ( $p<0.05$ , ANOVA post-hoc analysis). The iAUC of triglycerides in VLDL1 was significantly reduced with diet D compared to the control diet ( $p<0.05$ ).

**Conclusion:** These preliminary results indicate that diets rich in n-3 fatty acids and/or polyphenols positively influence the postprandial response of triglyceride-rich lipoproteins, and therefore may have favourable effects on cardiovascular risk.

Clinical Trial Registration Number:01154478

Supported by: European Community's Seventh Framework

## 879

### Determination of glycaemic index of corn flakes, white bread & ham, yogurt, muesli cake, fruit muesli and multigrain cake at different times of day using continuous glucose monitor

R. Chlup<sup>1</sup>, J. Zapletalova<sup>2</sup>, P. Kudlova<sup>3</sup>, V. Milata<sup>4</sup>, P. Seckar<sup>1</sup>, K. Peterson<sup>1</sup>, J. Matouskova<sup>1</sup>, J. Tomcalova<sup>1</sup>, J. Bartek<sup>5</sup>

<sup>1</sup>Dept. of Physiology, Faculty of Medicine, Palacký University Olomouc, Czech Republic, <sup>2</sup>Dept. of Biophysics, Faculty of Medicine, Palacký University Olomouc, Czech Republic, <sup>3</sup>Faculty of Humanities, Bata University, Zlin, Czech Republic, <sup>4</sup>St. Elizabeth University College of Health and Social Work Bratislava, Slovakia, <sup>5</sup>Faculty of Medicine, Palacký University Olomouc, Czech Republic.

**Background and aims:** Glycaemic index of foods (GI) is routinely determined according to the ISO 26642:2010 in at least 10 healthy persons after an overnight fast using fingerprick or venous blood analysed in the laboratory. The objective of this prospective open label study was (1) to assess the differences of GI for 6 popular foods determined by means of continuous glucose monitoring system (CGMS) with transcutaneous sensor, transmitter and monitor at breakfast, lunch, snack and two dinner times and (2) to assess the input of CGMS and related software for GI determination.

**Materials and methods:** In November 2011, 19 healthy volunteers aged 18–44 y, BMI  $23.5 \pm 1.1$  kg/m<sup>2</sup> (mean  $\pm$  SE), CGMS-trained, completed the study. Each subject consumed 6 different foods and glucose standard (50 g), each of them on 5 separate occasions at 5 different times of day according to a defined meal plan over a 9-day period. There were no tests on day 4 which was used to recharge the transmitter; tested persons were allowed to consume foods according to their free choice. Daily energy intake varied mostly between 7 000 and 8 000 kJ (250 g of sacharides, 80 g of proteins, up to 50 g of fat). The sensor was calibrated 3 times a day using glucometer. Any tests that did not fulfill the evaluation criteria (at least 210 min fast before and after meal start, exact portion of food consumed within 30 min, sensor failures exceeding 25 min) were not processed. GIs exceeding 3 times the interval between 25 and 75 percentil were excluded, such as 509/665 tests (77%, namely 69–85% of tests with each individual food) were analyzed using software SPSS v. 15.0. Data were processed using the software Carelink and DegifXL5.

**Results:** In all individual foods the Shapiro-Wilk test showed a non-normal distribution of GI. Therefore the values are presented as medians and percentil 25 and 75. The paired Wilcoxon signed-rank test with Bonferroni correction revealed no significant differences between respective GIs determined at breakfast, lunch, snack and dinner times. Median of individual means is the final GI (Table 1).

**Conclusion:** Tests performed at different times of day using CGMS appear to be a promising method for GI determination. Presenting the results as medians and percentiles brings more information on distribution of GI values. Despite of deviations from ISO 26642 this approach offers a better insight into postprandial evolution of glycaemia over 24 h.

Table 1: GI of foods [%] at different times of day (median, percentil 25, 75); median of means

Meal	Breakfast	Lunch	Snack	Dinner1	Dinner2	Means
Time	7.00	11.00	15.00	19.00	23.00	
Dark choco	33	46	101	80	40	60
cornies 85 g	18-98	31-94	74-124	26-139	16-96	51-91
White bread	76	43	108	89	87	81
& ham 240 g	65-119	31-63	39-209	28-114	38-108	52-106
Flavoured	37	39	25	35	32	41
yogurt 300 g	25-86	31-48	12-50	23-78	9-47	30-57
Muesli cake 75 g	57	69	80	73	64	65
	45-76	41-87	27-92	49-102	22-82	56-91
Fruit muesli	37	57	68	64	42	57
& milk 100 ml	17-86	11-99	26-98	37-78	34-59	41-66
Multigrain	53	33	66	63	43	57
cake 65 g	23-82	24-53	46-93	41-100	21-81	44-82
Glucose 50 g	75	113	104	90	92	100
	52-128	71-120	75-126	67-133	75-142	

Supported by: Faculty of Medicine, UP Olomouc

## 880

**Does treatment with n-3 polyunsaturated fatty acids influence vitamin D status in severely obese non-diabetic subjects?**T.M. Stulnig<sup>1,2</sup>, L. Leitner<sup>1,2</sup>, R. Marculescu<sup>3</sup>, M. Zeyda<sup>1,2</sup>, B.K. Itariu<sup>1,2</sup>;<sup>1</sup>Clinical Division of Endocrinology and Metabolism, Medical University of Vienna, <sup>2</sup>Christian Doppler Laboratory for Cardio-Metabolic Immunotherapy, <sup>3</sup>Department of Laboratory Medicine, Medical University of Vienna, Austria.

**Background and aims:** Hypovitaminosis D is frequent among obese individuals. Recently, it was shown that the vitamin D status of patients receiving vitamin D3 supplements correlated negatively with the amount of total ingested polyunsaturated fatty acids (PUFA). n-3 PUFA and vitamin D have immunomodulatory effects which could help prevent obesity-associated complications, such as cardiovascular disease. Thus, we aimed to investigate whether treatment with long chain n-3 PUFA negatively affects vitamin D status in obese patients.

**Materials and methods:** In a randomized, controlled trial we treated 55 severely obese (BMI ≥ 40 kg/m<sup>2</sup>) non-diabetic patients with either 3,3 g/d n-3 PUFA (EPA/DHA) or the same amount of butter fat as control for 8 weeks. At baseline and end of treatment we determined plasma fatty acid profiles, circulating inflammatory marker and serum 25-hydroxyvitamin D (25(OH)D) concentrations. Statistical analysis was performed using RM-ANOVA and Spearman's rank correlation.

**Results:** At baseline, 44/55 patients were vitamin D deficient (25(OH)D ≤ 50 nmol/l). The serum 25(OH)D concentration correlated positively with plasma EPA content ( $\rho = 0,30$ ,  $P = 0,03$ ) and negatively with both IL-6 and hsCRP serum concentration ( $\rho = -0,31$ ,  $P = 0,02$  and  $\rho = -0,29$ ,  $P = 0,03$ , respectively). Overall, treatment with n-3 PUFA did not significantly affect vitamin D status compared to control ( $P = 0,57$ ). However, n-3 PUFA treatment during summer months significantly increased serum 25(OH)D concentrations ( $P = 0,002$ ). Median serum triglyceride and IL-6 concentrations were decreased by 15,9% and 24,8% in the n-3 PUFA compared to control group, respectively, but correlations of 25(OH)D with EPA and inflammatory markers were no longer detectable at the end of n-3 PUFA treatment.

**Conclusion:** Vitamin D status of severely obese patients does not deteriorate by 8 week n-3 PUFA treatment as has been suggested by epidemiological data. Potential synergistic effects of n-3 PUFA and vitamin D on obesity-associated comorbidities remain to be elucidated.

Clinical Trial Registration Number: NCT00760760

Supported by: ÖNB, P12735; European Community/FP7 #201608; Christian Doppler Society

## 881

**Animal protein intake during puberty is related to the IGF-axis and insulin sensitivity in young adulthood**A.E. Buyken<sup>1</sup>, T. Remer<sup>2</sup>, K.E. Assmann<sup>2</sup>, D. Krupp<sup>2</sup>, G. Cheng<sup>2,3</sup>, S. Wudy<sup>3,4</sup>, A.L.B. Günther<sup>4</sup>, G. Joslowski<sup>2</sup>;<sup>1</sup>Department of Nutritional Epidemiology - DONALD, Rheinische-Friedrich-Wilhelms-University Bonn, <sup>2</sup>Department of Nutritional Epidemiology - DONALD, Rheinische-Friedrich-Wilhelms-University Bonn, <sup>3</sup>Center of Child and Adolescent Medicine, Laboratory for Translational Hormone Analytics in Paediatric Endocrinology, Gießen, <sup>4</sup>Department of Nutritional, Food, and Consumer Sciences, Fulda University of Applied Sciences, Germany.

**Background and aims:** Evidence from observational studies suggests that higher intakes of animal protein may predispose to some types of cancer and type 2 diabetes. This study addressed the hypothesis that such relations may already emerge during adolescence, analyzing animal and vegetable protein intakes during puberty for prospective associations with the IGF-axis and insulin sensitivity in young adulthood.

**Materials and methods:** Multivariable regression analysis was performed on data from 213 (118 female and 95 male) DONALD participants with at least two plausible 3-day weighed dietary records during puberty (girls: 9-14 years, boys: 10-15 years) and one blood sample in young adulthood (18-35 years). Mean levels of IGF-1, IGFBP-3 (n=213) as well as IGFBP-1, IGFBP-2, and HOMA-IR index (n=201) were compared between tertiles of habitual pubertal animal and vegetable protein intake.

**Results:** Among women, a habitually higher animal protein intake during puberty was independently associated with higher levels of IGF-1 (ptrend=0.009) and IGFBP-3 (ptrend=0.04), lower IGFBP-2 (ptrend=0.1),

and higher HOMA-IR values (ptrend=0.048), but not with IGFBP-1 (ptrend=0.5) in young adulthood. IGF-1 values in energy-adjusted tertiles of animal protein were 195 (95% CI 166, 227), 244 (211, 279) and 229 (197, 263) ng/ml, adjusted for age, blood sample, early life, socioeconomic and nutritional factors. Mean adjusted HOMA-IR values were 2.2 (1.9, 2.5), 2.7 (2.4, 3.1) and 2.7 (2.4, 3.1). Additional consideration of baseline fat mass attenuated the relation to HOMA-IR (ptrend=0.099), but not that to IGF-1 (ptrend=0.005). No prospective associations were observed for intakes of vegetable protein (ptrend>0.4). Protein intakes were not related to the IGF-axis or HOMA-IR among men.

**Conclusion:** Our data suggest that, among women, a habitually higher animal protein intake during puberty may precipitate an up-regulation of the IGF-axis and a modest adaptive decrease in insulin sensitivity, which are both still discernible in young adulthood.

Supported by: MIWF, WCRF (no 2010/248)

## 882

**Prevalence of gestational diabetes by WHO and IADPSG criteria in women with four major dietary patterns: The STORK Groruddalen study**C. Sommer<sup>1</sup>, K. Mørkrid<sup>1,2</sup>, A. Mosdøl<sup>3</sup>, L. Sletner<sup>4,2</sup>, A.K. Jenum<sup>5</sup>, K.I. Birkeland<sup>1,2</sup>;<sup>1</sup>Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Aker, <sup>2</sup>Institute of Clinical Medicine, University of Oslo, <sup>3</sup>Institute of Health, Nutrition and Management, Oslo and Akershus University College of Applied Sciences, <sup>4</sup>Norwegian Resource Centre for Women's Health, Oslo University Hospital, Rikshospitalet, <sup>5</sup>Institute of Health and Society, University of Oslo, Norway.

**Background and aims:** Gestational Diabetes Mellitus (GDM) affects short and long term health of both mother and offspring. Diet is thought to be an important factor affecting GDM risk and prevention. The aim was to explore how dietary patterns were associated with prevalence of GDM diagnosed by WHO and the The International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, and to assess the relation with ethnicity.

**Materials and methods:** STORK Groruddalen is a population-based prospective cohort study of 823 (74% of the invited, 59% of non-Western origin) healthy pregnant women and their offspring attending three primary antenatal clinics in Eastern Oslo. The study population comprised 757 pregnant women who completed an interview administered food frequency questionnaire in 28 weeks of gestation. A hierarchical cluster analysis using squared euclidian distance was conducted to derive dietary patterns. The stability of the clusters was examined through split half replication. The dietary patterns were examined for association with GDM by the two criteria and ethnicity. All statistical analyses were performed using IBM SPSS Statistics 19.

**Results:** Four robust clusters were identified. Women born in Norway or other western countries were mainly associated with cluster 4 (60.5%), while women with other origin were rather evenly assigned into all four clusters. Cluster 1 and 2 had the highest prevalence of GDM (17.2 and 16.4 %), while cluster 3 and 4 had the lowest prevalence of GDM (9.9 and 10.1 %) with the WHO criteria. With the IADPSG criteria, clusters 1-3 had a similar prevalence of GDM (35.2-36.7 %) whereas cluster 4 had a significant lower prevalence (23.7 %,  $P = 0.008$ ). Except from for cluster 3, the prevalence trend were quite similar across the two criteria. A non-significant trend towards lower prevalence of GDM by IADPSG criteria in cluster 4 still was present when adjusting for ethnic background ( $p_{Western} = 0.493$ ,  $p_{Non-Western} = 0.671$ ), but the power to detect that was limited due to small numbers.

**Conclusion:** These results may indicate that dietary patterns similar to cluster 3 - high in beverages with sugar, polished rice and regular pasta, and low in fruit, vegetables, fish and milk - is associated with GDM with the IADPSG criteria, but not hyperglycemia severe enough to be identified as GDM with the WHO criteria. A diet similar to cluster 4 - high in whole grain/unpolished products, fruit and vegetables and low fat milk - may be more beneficial with respect to reduce the prevalence of GDM and should be tested in randomized studies.



**Table 1.** Prevalence of GDM by WHO and IADPSG criteria by ethnic origin within the dietary patterns

Ethnic origin	Prevalence of GDM, n (%)	Cluster 1 (n=125)	Cluster 2 (n=199)	Cluster 3 (n=183)	Cluster 4 (n=250)	p*
Western	WHO criteria	0 (0)	8 (15.1)	6 (10.0)	19 (10.2)	0.524
	IADPSG criteria	2 (22.2)	15 (28.3)	18 (30.0)	40 (21.4)	0.493
Non Western	WHO criteria	21 (18.6)	24 (16.9)	12 (9.9)	6 (9.8)	0.150
	IADPSG criteria	43 (37.4)	56 (39.7)	46 (37.7)	19 (30.6)	0.671
All	WHO criteria	21 (17.2)	32 (16.4)	18 (9.9)	25 (10.1)	0.062
	IADPSG criteria	45 (36.3)	73 (36.7)	64 (35.2)	59 (23.7)	0.008

\*  $\chi^2$  test

Supported by: South-Eastern Norway Regional Health Authority

## 883

### Fatty liver and insulin resistance: time course appearance of the changes induced by a fructose rich diet

L.M. Massa, C. Blaiotta, M.C. Castro, J.J. Gagliardino, F. Francini;  
CENEXA Center of Experimental and Applied Endocrinology, National University of La Plata, Argentina.

**Background and aims:** We have previously shown that administration of fructose to normal rats during 3 weeks induced insulin resistance, impaired glucose tolerance, fatty liver and hepatic carbohydrate metabolism abnormalities. The aim of this study was to evaluate the time-course of development of these alterations triggered by a fructose rich diet (FRD).

**Materials and methods:** For this purpose we fed normal male Wistar rats for 7, 14 and 21 days with a) a standard commercial diet (C) and b) a C diet plus 10% fructose in the drinking water (DRF). At each time we measured glycemia (G), triglyceridemia (TG) and insulinemia (I). HOMA-IR, HOMA- $\beta$  and Index for hepatic sensitivity to insulin (IHIS) ratios were calculated. In liver we measured a) protein carbonyl groups and reduced glutathione (GSH) as oxidative stress markers (OSM), b) liver glycogen and triglyceride content, c) glucokinase (GK), glucose-6-phosphatase (G-6-Pase) and glucose-6-phosphate dehydrogenase (G-6-P DH) activities and d) SREBP-1c, fatty acid synthase (FAS), glycerol-3-phosphate acyltransferase (GPAT), G-6-Pase and G-6-P DH gene expression.

#### Results:

	C1	FRD1	C2	FRD2	C3	FRD3
In (ng/ml)	0,5 $\pm$ 0,07	0,6 $\pm$ 0,06	0,6 $\pm$ 0,06	0,7 $\pm$ 0,06	0,6 $\pm$ 0,04	1,1 $\pm$ 0,2*
G (mg/dl)	115 $\pm$ 3	118 $\pm$ 4	103 $\pm$ 3	121 $\pm$ 6	117 $\pm$ 2	117 $\pm$ 3
TG (mg/dl)	103 $\pm$ 6	134 $\pm$ 11*	112 $\pm$ 15	172 $\pm$ 15*	102 $\pm$ 11	226 $\pm$ 31*
IHIS (k/GPA $\times$ lnPA)	5,07 $\pm$ 0,9	4,12 $\pm$ 0,4	4,72 $\pm$ 0,7	3,44 $\pm$ 0,3	4,15 $\pm$ 0,26	2,26 $\pm$ 0,3*
HOMA IR	3,8 $\pm$ 0,5	4,9 $\pm$ 0,4	3,9 $\pm$ 0,3	5,2 $\pm$ 0,5*	4,6 $\pm$ 0,3	8,1 $\pm$ 1,2*
HOMA $\beta$	39,4 $\pm$ 5	47,2 $\pm$ 5,2	45,8 $\pm$ 5,3	53,5 $\pm$ 5,2	48,5 $\pm$ 3,5	78,5 $\pm$ 11,6*
GK (mU/mg prot)	10 $\pm$ 0,5	12 $\pm$ 0,4*	9 $\pm$ 0,8	16 $\pm$ 1*	9 $\pm$ 0,6	19 $\pm$ 1*
G6Pase (latency %)	8 $\pm$ 2	13 $\pm$ 3	8 $\pm$ 1	18 $\pm$ 2*	7 $\pm$ 1	15 $\pm$ 1*
Glycogen ( $\mu$ g/mg t)	7,0 $\pm$ 0,4	7,4 $\pm$ 0,3	6,8 $\pm$ 0,4	8,2 $\pm$ 0,2*	8,4 $\pm$ 0,3	10,8 $\pm$ 0,4*
Liver TG ( $\mu$ g/100 mg t)	304 $\pm$ 13	470 $\pm$ 59*	277 $\pm$ 30	376 $\pm$ 30	301 $\pm$ 25	469 $\pm$ 17*

(\*p&lt;0.05 vs.C)

Enhanced expression of all genes was recorded after one week. Carbonyl content was increased and GSH was reduced at two weeks treatment.

**Conclusion:** Increased serum and liver content of TG were the earliest changes induced by fructose administration while OSM and other liver dysfunctions appeared on the second week while enhanced HOMA  $\beta$  and hyperinsulinemia ( $\beta$  cell reaction) were the latest change recorded suggesting that would be the consequence of metabolic and OS occurred in liver and probably adipose tissue as well.

Supported by: PIP 0371 CONICET

## 884

### Comparison of glycaemia variability in patients with type 2 diabetes given mitiglinide or sitagliptin, using continuous glucose monitoring: a pilot study

K. Ando, R. Nishimura, D. Tsujino, C. Seo, A. Morimoto, M. Sakamoto, K. Utsunomiya;  
Jikei University School of Medicine, Tokyo, Japan.

**Background and aims:** This study aimed to compare glucose variability in patients with type 2 diabetes given Mitiglinide (M) or Sitagliptin (S), using continuous glucose monitoring (CGM).

**Materials and methods:** The present study included nine patients with type 2 diabetes, aged from 20 to 79 years of age. The patients were randomly assigned to receive either M 30 mg/day or S 50 mg/day. After two months of treatment with M or S, they were hospitalised for four days, for evaluation by CGM. When the patients were discharged from the hospital, the drugs they received were switched so that they then took the one that they had not previously had. Again, after two months of treatment, they were hospitalised for four days for evaluation by CGM. The patients were given the same meals on days two and three of both their hospitalisations, and the CGM data on day three were used. Variables that were compared by using t-test included 1) the mean glucose levels, 2) the standard deviations (SD) of mean glucose levels, 3) the mean amplitude of glycaemic excursions (MAGE), 4) the range of glucose increase from pre-prandial to post-prandial peak glucose levels, 5) the time from pre-prandial to post-prandial peak glucose levels, and 6) the area under the curve (AUC) that measured over 140 mg/dL three hours after each meal. Statistical analyses were performed using SPSS 17.0. This study was approved by the Institutional Review Board of our university.

**Results:** The patients were  $59.8 \pm 10.4$  (mean  $\pm$  SD) years old, had HbA1c value  $7.29 \pm 0.85\%$  and BMI of  $24.7 \pm 3.20$  kg/m<sup>2</sup>. Their mean glucose levels tended to be lower with M than with S (mean glucose levels, M  $147.4 \pm 26.5$  vs. S  $158.6 \pm 34.2$  mg/dL; SD, M  $24.7 \pm 11.5$  vs. S  $30.1 \pm 14.4$ ), while MAGE were found to be similar with M and S ( $77.3 \pm 34.8$  and  $84.7 \pm 40.4$ , respectively). The range of the glucose increase from pre-prandial to post-prandial peak glucose levels was also similar for M and S. The time to the post-prandial peak glucose levels after breakfast, lunch, and dinner, with M and S, was  $102.2 \pm 33.6$  and  $108.9 \pm 37.1$  minutes,  $77.8 \pm 31.6$  and  $110.0 \pm 27.6$  minutes, and  $78.3 \pm 26.5$  and  $92.2 \pm 23.7$  minutes, respectively. This shows that the time from the pre-prandial to the post-prandial peak glucose levels was significantly ( $P = 0.022$ ) shorter after lunch, with M than with S, but was similar with M or S after both breakfast and dinner. The AUC measuring over 140 mg/dL three hours after breakfast tended to be lower with M than with S ( $4547.2 \pm 3682.0$  and  $8005.6 \pm 6815.2$ , respectively,  $P = 0.059$ ) but, at three hours after lunch or dinner, there was no significant difference between M or S.

**Conclusion:** An in-depth CGM-based study of glucose variability with M and S showed that there was no significant difference between M and S with respect to the SD, MAGE, and the range of glucose increase from the pre-prandial to the post-prandial peak glucose levels. The time from the pre-prandial to the post-prandial peak glucose levels was shown to be significantly shorter with M than with S after lunch, and the mean glucose levels and the AUC above 140 mg/dL three hours after breakfast tended to be lower with M than with S.

Clinical Trial Registration Number: UMIN000005517

Supported by: Japan Diabetes Foundation

## 885

### Dietary intake and serum level of n-3 polyunsaturated fatty acids as predictors of DPP-4 inhibitor efficacy in patients with type 2 diabetes

M. Iwasaki<sup>1</sup>, D. Yabe<sup>2</sup>, F. Hoshian<sup>1</sup>, T. Tsuji<sup>1</sup>, N. Hirose<sup>1</sup>, T. Matsumoto<sup>1</sup>, N. Kitatani<sup>1</sup>, K. Sugawara<sup>2</sup>, R. Usui<sup>2</sup>, H. Kuwata<sup>2</sup>, S. Fujiwara<sup>2</sup>, K. Watanabe<sup>2</sup>, T. Hyo<sup>2</sup>, T. Kurose<sup>2,1</sup>, Y. Seino<sup>2</sup>;

<sup>1</sup>Division of Metabolism and Clinical Nutrition, <sup>2</sup>Division of Diabetes, Clinical Nutrition and Endocrinology, Kansai Electric Power Hospital, Osaka, Japan.

**Background and aims:** Dipeptidyl peptidase-4 (DPP-4) inhibitors improve glycemic control in patients with type 2 diabetes by preventing degradation of two incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are secreted from the intestine upon ingestion of various nutrients. Recent studies have shown associations of DPP-4 inhibitor efficacy with age and fasting plasma glucose levels as well as with baseline HbA1c and BMI, but clinical parameters for predicting

the outcome of DPP-4 inhibitor treatment are largely unknown. This study was initiated to identify clinical and dietary parameters for predicting efficacy of DPP-4 inhibitors.

**Materials and methods:** A total of 72 untreated patients with type 2 diabetes who received monotherapy of a DPP-4 inhibitor (sitagliptin, alogliptin or vildagliptin) for 4 months were examined for changes in HbA1c and BMI and self-administered 3-day food records and serum levels of fatty acids. Multiple linear regression analyses were performed to identify parameters associated with HbA1c reduction and simple regression analyses were carried out to evaluate their contributions.

**Results:** DPP-4 inhibitors, as in previous reports, significantly reduced HbA1c levels but not body weight [Before  $7.2 \pm 0.7\%$ ; 4 months after initiation  $6.7 \pm 0.6\%$  (paired t-test,  $p < 0.01$  versus before)]. Multiple regression analysis of HbA1c reduction taking into account sex, age, duration of diabetes, BMI, baseline HbA1c and estimated intake of various food categories in 3-day food records, revealed that HbA1c reduction was well correlated with baseline HbA1c but was not correlated with BMI. HbA1c reduction also showed a significant association with estimated intake of fish in the food records. The beneficial effects of fish on human health have been partly attributed to n-3 polyunsaturated fatty acids (PUFA) such as EPA and DHA. We therefore investigated the association of HbA1c reduction with estimated intake of PUFA. HbA1c reduction was significantly correlated with estimated intake of EPA and DHA along with baseline HbA1c. Since serum EPA and DHA levels might serve as markers for intake of corresponding fatty acids, we analyzed the associations of HbA1c reduction with serum EPA and DHA levels. Serum levels of EPA and DHA, but not those of n-6 PUFA arachidonic acid, were well correlated with HbA1c reduction.

**Conclusion:** HbA1c reduction by DPP-4 inhibitors is significantly associated with estimated dietary intake of fish and n-3 PUFA and also with serum levels of EPA and DHA. Thus, the clinical efficacy of DPP-4 inhibitors can be predicted by serum EPA and DHA levels.

## PS 071 Novel unconventional therapies

### 886

#### Vitamin D supplementation as an adjuvant therapy for Saudi Arabian patients with type 2 diabetes mellitus: an 18-month interventional study

A.-D. Nasser, Biomarkers Research Program and Prince Mutaib Chair for Biomarkers of Osteoporosis;  
King Saud University, Riyadh, Saudi Arabia.

**Background and aims:** Vitamin D deficiency has been shown to impair human insulin action, suggesting a role in the pathogenesis of Type 2 diabetes mellitus (T2DM). Despite the level of sunshine in Saudi the population has significant vitamin D deficiency based on our previous studies. In this prospective interventional study we investigated the effect of vitamin D (Vit D) supplementation in the metabolic and glycemic profiles of the Saudi T2DM subjects pre and post supplementation to improve known Vit D deficiency compounded in metabolic states such as T2DM.

**Materials and methods:** T2DM Saudi subjects (men: age:  $56.6 \pm 8.7$  yr, BMI,  $n=34$ ;  $29.1 \pm 3.3$  kg/m<sup>2</sup>; females: Age:  $51.2 \pm 10.6$  yr, BMI  $34.3 \pm 4.9$  kg/m<sup>2</sup>;  $n=58$ ) were recruited and given 2000IU vitamin D3 daily for 18 months. Anthropometrics and biochemical data was collected (0, 6, 12, 18 months) to monitor serum 25 hydroxyvitamin D [25(OH) D (nmol/L)] using a commercial ELISA, as well as glycemic and lipid profiles.

**Results:** In all T2DM subjects there was a significant increase in mean circulating 25(OH)D levels from baseline ( $32.2 \pm 1.5$  nmol/L) to 18 months ( $54.7 \pm 1.5$  nmol/L;  $p < 0.001$ ) as well as with serum calcium [Baseline =  $2.3 \pm 0.23$  mmol/L versus 18 months =  $2.6 \pm 0.1$  mmol/L;  $p = 0.003$ ]. A significant increase in HDL-cholesterol was noted only in females ( $p < 0.001$ ), as well as a significant decrease in LDL- calcium [Baseline =  $4.4 \pm 0.8$  mmol/L versus 18 months =  $3.6 \pm 0.8$  mmol/L] and total cholesterol calcium [Baseline =  $5.4 \pm 0.2$  mmol/L versus 18 months =  $4.9 \pm 0.3$  mmol/L] ( $p < 0.001$  and  $p < 0.001$  respectively). In all subjects, glycemic parameters (glucose, insulin, HOMA-IR), blood pressure and BMI were comparable.

**Conclusion:** In summary, the results highlight that despite oral Vitamin D supplementation (2000IU/day) in Saudi subjects with T2DM; circulating 25(OH)D levels still remain deficient by 22% below normal 18 months post treatment. However, supplementation appeared to significantly improve lipid profile with a change in HDL/LDL ratio which was more pronounced in T2DM females, offering other benefits for health. This study suggests that T2DM Saudi subjects require a higher Vitamin D supplementation (3000IU/day) as a clinical recommendation to achieve normal vitamin D status.

Supported by: King Abdulaziz City for Science and Technology (KACST AT-29-38)

### 887

#### The novel oral drug subetta exerts anti-diabetic effect in the diabetic GK/Par rat

E.A. Gorbunov<sup>1</sup>, S.A. Tarasov<sup>1</sup>, O.I. Epstein<sup>1</sup>, D. Bailbe<sup>2</sup>, E. Philippe<sup>2</sup>, B. Portha<sup>2</sup>;

<sup>1</sup>OOO NPF Materia Medica Holding, Moscow, Russian Federation,

<sup>2</sup>B2PE-BFA Unit, University Paris-Diderot, Paris, France.

**Background and aims:** The search for new targets and the development of novel anti-diabetic drugs ensuring high efficacy on a background of minimal side effects are still pressing issues. Subetta, containing ultra diluted solution of antibodies to C- terminal fragment of insulin receptor and antibodies to endothelial NO synthase, could be one of such drugs. Preliminary in-house studies in rats with streptozotocin-induced diabetes suggested that Subetta reduced hyperglycemia to an extent similar to Rosiglitazone. Toxicological studies revealed a high drug safety. The aim of the present pre-clinical study was to evaluate Subetta antidiabetic efficacy in a rat model of non-obese type 2 diabetes.

**Materials and methods:** The study was conducted in adult male Goto Kaki-zaki rats from the Paris colony (GK/Par) (age 10-12 weeks). The rats were randomized into 3 groups ( $n=12$  each): group 1 - Subetta (5 ml/kg/daily), group 2 - Rosiglitazone (5 mg/kg/daily; reference drug), group 3 (control) - vehicle (5 ml water/kg/daily). The drugs and control were administered by gavage for 28 days once daily (9:00). Twice a week (non fed state) prior to drug administration (9:00), the animals were weighted and water, food intake

and basal plasma glucose level were evaluated. One day prior to first drug administration (D0), 3 hours after the first drug administration (12:00) (D1), and 3 hours after the last drug administration (12:00) (D28), oral glucose tolerance tests (OGTT) (2 g glucose/kg rat; unanesthetized state) were sequentially performed on each animal. Plasma glucose and insulin changes were determined from blood samples collected before and 5, 10, 15, 30, 60 and 120 min after glucose administration. Glucose AUC and insulin AUC were calculated to quantify glucose tolerance and glucose-induced insulin secretion. Non-parametric Mann-Whitney U and Wilcoxon tests were used for statistical analysis of the results.

**Results:** At D28, basal plasma glucose levels were significantly decreased in Subetta and Rosiglitazone groups as compared to control group:  $147 \pm 4$  mg/dL ( $p < 0.01$ ) and  $145 \pm 4$  mg/dL ( $p < 0.01$ ) and  $165 \pm 4$  mg/dL, respectively. The OGTT data showed that Subetta administration (similar to Rosiglitazone) prevented significantly ( $p < 0.01$ ) the age-related (from D0 to D28) spontaneous deterioration of glucose tolerance as seen in the control group: from D0 to D28, glucoseAUC increased by 47% in the control group, and only by 17% and 27% in the Subetta and Rosiglitazone groups respectively. Subetta did not significantly modified the glucose-induced insulin secretion which remained as low on D28 as it was on D0. Subetta and Rosiglitazone did not affect body weight, despite a slight reduction in daily food intake as compared to control group: respectively,  $66 \pm 1$  g food/kg rat ( $p < 0.05$ ) and  $62 \pm 1$  g food/kg rat ( $p < 0.01$ ) and  $71 \pm 1$  g food/kg rat.

**Conclusion:** Chronic administration of the novel oral drug Subetta improves glucose control in the GK/Par rat model of type2 diabetes, to an extent similar to that of Rosiglitazone. This cannot be explained by insulin secretion restoration and we hypothesize that Subetta mostly acts via an insulin sensitizing effect upon target tissues.

## 888

### Testosterone replacement therapy in middle-aged to elderly hypogonadal men leads to continuous weight loss and reduction in waist circumference over five years

F. Saad<sup>1,2</sup>, A. Haider<sup>3</sup>,

<sup>1</sup>Scientific Affairs Men's Healthcare, Bayer Pharma AG, Berlin, Germany,

<sup>2</sup>Research Department, Gulf Medical University School of Medicine, Ajman, United Arab Emirates, <sup>3</sup>Private Urology Practice, Bremerhaven, Germany.

**Background and aims:** Obesity in men is frequently associated with testosterone deficiency. Adipose tissue is an active endocrine organ producing substances (e.g., estrogens, cortisol, leptin, inflammatory cytokines) which suppress the production of endogenous testosterone at the hypothalamic-pituitary level and at the testicular level. This study aimed at investigating the effects of testosterone replacement in hypogonadal men on parameters of obesity.

**Materials and methods:** Open-label, single-center, cumulative, prospective registry study of 255 men (mean age  $60.6 \pm 8.0$  years), with testosterone levels  $\leq 3.5$  ng/mL (12 nmol/L) receiving parenteral testosterone undecanoate 1000 mg at baseline, after 6 weeks and thereafter at 12-week intervals.

**Results:** After a maximum treatment duration of five years, the following changes occurred: testosterone increased from  $2.87 \pm 0.4$  to  $5.26 \pm 0.44$  ng/mL. Body weight (kg) decreased from  $106.22 \pm 16.93$  (minimum: 70, maximum: 139) to  $90.07 \pm 9.51$  (min 74.00, max 115). The statistical significance was  $p < 0.0001$  vs baseline and vs the previous year over 5 years indicating a continuous weight reduction. Waist circumference (cm) declined from  $107.24 \pm 9.14$  (min 86, max 129) to  $98.46 \pm 7.39$  (min 84, max 117) ( $p < 0.0001$  vs baseline and vs the previous year over 5 years). Body mass index (BMI, m/kg<sup>2</sup>) declined from  $33.93 \pm 5.54$  (min 21.91, max 46.51) to  $29.17 \pm 3.09$  (min 22.7; max 36.71) ( $p < 0.0001$  vs baseline and vs the previous year over 5 years). The mean per cent weight loss after 1 year was  $4.12 \pm 3.48\%$ , after 2 years  $7.47 \pm 5.01\%$ , after 3 years  $9.01 \pm 6.5\%$ , after 4 years  $11.26 \pm 6.76\%$  and after 5 years  $13.21 \pm 7.24\%$ . At baseline, 96% of men had a waist circumference of  $\geq 94$  cm. This proportion decreased to 71% after 5 years.

**Conclusion:** The normalization of testosterone in hypogonadal men resulted in progressive loss of weight, waist circumference and BMI over the full 5 years of the study.

Supported by: Bayer Pharma AG

## 889

### TR-beta selective thyromimetics (KB-141) stimulates the proliferation of insulin secreting beta cells

T. Kim<sup>1,2</sup>, J. Lee<sup>1</sup>, H. Jung<sup>1</sup>, N. Han<sup>1</sup>, S. Kim<sup>1</sup>, E. Lee<sup>1</sup>, T. Kim<sup>1</sup>, M. Kwon<sup>1</sup>, S. Lee<sup>1</sup>, M. Kim<sup>1,2</sup>, M. Park<sup>3</sup>, B. Rhee<sup>1,2</sup>, G. Grover<sup>4</sup>, J. Park<sup>1</sup>;

<sup>1</sup>Division of Endocrinology and Metabolism, Department of Internal Medicine, Pusan Paik Hospital, Inje University, Busan, Republic of Korea,

<sup>2</sup>Paik Institute for Clinical Research, Busan, Republic of Korea, <sup>3</sup>Department of Internal Medicine, Dong-A University, Busan, Republic of Korea,

<sup>4</sup>Department of Pharmacology, Eurofins-Drug Safety Laboratories, Dayton, USA.

**Background and aims:** Thyroid hormone (T3) influences a variety of physiological processes, including cell growth, differentiation and metabolisms in mammals. Recent studies have indicated that all these effects are mediated by the growth stimulatory effect of T3 to the thyroid hormone nuclear receptors (TRs). Recent studies have reported that the pancreatic  $\beta$  cells also express TRs. KB-141, the TR $\beta$ -selective thyromimetics would show its favorable benefits on  $\beta$ -cells without untoward systemic side effects shown in thyroid hormone. The aim of our study is to clarify the effects of KB-141 on the proliferation of insulin secreting  $\beta$  cells by KB-141.

**Materials and methods:** RINm5, INS-1 cells and isolated primary rodent islets (SD rats) were used in our experiments. Triiodothyronine (T3) and KB-141 were applied to cultured cell lines and isolated primary rodent islets in various concentrations and time intervals along with vehicle only control. At 24, 48 and 72 h of continuous exposure, viable cells were harvested and counted. In isolated rodent islets, Ki-67, insulin and glucagon counter-staining were performed. TR $\beta$ 1 expression, viability, proliferation were analyzed by Western blot, RT-PCR, CCK-8, BrDU, FACS, TUNNEL assay and Trypan Blue negative cell numbers in a Thomas's hemacytometer. Insulin secretory function of exposed  $\beta$ -cells were verified by the amount of insulin mRNA and GSIS.

**Results:** T3 and KB-141 significantly promoted the growth of RINm5, INS-1 cells, and the  $\beta$ -cells in isolated primary islets and improved glucose stimulated insulin secretion.

**Conclusion:** KB-141 would be used as a fundamental diabetes treatment to increase functioning  $\beta$ -cell mass without untoward systemic side effects caused by non-specific thyroid hormone receptor stimulation.

## 890

### Kimchi has favourable effects on metabolic parameters and cytokine levels in patients with type 2 diabetes mellitus

S.-Y. An, M. Lee, J. Jeon, S. Han, H. Kim, D. Kim, K.-W. Lee;

Department of Endocrinology and Metabolism, Ajou University School of Medicine, Suwon, Republic of Korea.

**Background and aims:** Kimchi is a traditional fermented Korean food and it has drawn world-wide interest due to its various beneficial effects. This study demonstrated the beneficial effects of Kimchi on metabolic parameters, cytokine levels, and glucose levels in patients with T2DM.

**Materials and methods:** Twenty patients with T2DM were randomly assigned to two 4-week diet phases, separated by a 2-week washout period (cross-over design). During each diet phase, the subjects consumed portions of either fresh or fermented Kimchi, which were created from the same standardized recipe and ingredients, but differed in terms of fermentation. One-day-old and 10-day-old Kimchi were defined as "fresh" and "fermented" Kimchi, respectively. Diet composition, anthropometric data, glucose levels, lipid parameters, and cytokines levels were analyzed.

**Results:** Fiber intake was significantly increased after ingestion of either type of Kimchi. Diastolic blood pressure decreased significantly from  $73.3 \pm 12.1$  mmHg to  $68.1 \pm 9.5$  mmHg in the fermented Kimchi group. Hemoglobin A1c percentage showed a significant decrease after ingestion of fresh Kimchi from  $6.76 \pm 0.58\%$  to  $6.59 \pm 0.53\%$ . Levels of fibroblast growth factor-21 (FGF21), known to be elevated in patients with T2DM due to FGF-21 resistance, decreased, and levels of adiponectin increased, after ingestion of fermented Kimchi ( $P < 0.05$ ).

**Conclusion:** Kimchi (fresh or fermented) had positive effects on diastolic blood pressure, hemoglobin A1c percentage, levels of metabolic cytokine including FGF-21, and adiponectin levels in patients with T2DM.



## 891

**Effect of natural vanadium-containing Jeju ground water on blood glucose in diabetes patients: a double-blind, randomised controlled trial**G. Koh<sup>1</sup>, D. Lee<sup>1</sup>, S. Lee<sup>1</sup>, E.-K. Kang<sup>1</sup>, H.-J. Han<sup>1</sup>, O.-K. Hwang<sup>1</sup>, S. Kim<sup>1</sup>, E.-J. Yang<sup>1</sup>, M.-K. Kim<sup>1</sup>, H.-J. Chin<sup>2</sup>;<sup>1</sup>Department of Internal Medicine, Jeju National University Hospital,<sup>2</sup>Department of Internal Medicine, Hankook General Hospital, Jeju, Republic of Korea.

**Background and aims:** Several studies have shown that vanadium supplementation enhanced insulin actions and lowered glucose levels in animals and small number of people. The Jeju ground water contains a high concentration of vanadium because Jeju Island is composed predominantly of basalts. We investigated the effect of Jeju ground water on blood glucose profile in patients with diabetes mellitus.

**Materials and methods:** We conducted a 12-week, prospective, double-blind, randomized controlled trial with intention-to-treat analysis. Total 196 patients drank a filtered tap water (C, n=67, vanadium 0 µg/L) transported from the outside of Jeju Island or one of two kinds of Jeju ground water (S1, n=66, vanadium 4–7 µg/L and S2, n=63, vanadium 24 µg/L). They had drunk 1 liter of test waters daily for 12 weeks.

**Results:** Baseline (BL) characteristics and parameters were not different among three groups. HbA1c tended to be decreased over time in S1 & S2 compared with C. However, it was not statistically significant. Fructosamines were significantly decreased at 12 week in S1 and 8 & 12 week in S2 more than C. Fasting plasma glucos (FPG) was significantly decreased at 8 week in S2 more than C (Table). All three groups did not show any significant adverse event

**Conclusion:** The Jeju ground water is likely to improve glucose level in diabetes people. In future, a nation-wide long-term clinical trial is needed to prove its glucose-lowering effect definitely.

Table. Blood glucose variables over time. \* p<0.05 vs C (adjusted for BL value).

Group	Variable	BL	4 week	8 week	12 week
C	HbA1c (%)	7.42±0.14	7.38±0.14	7.40±0.14	7.49±0.15
C	Fructosamine (µmol/L)	290±7	302±7	304±8	308±8
C	FPG (mg/dL)	139±4	143±5	142±4	142±4
S1	HbA1c (%)	7.43±0.13	7.31±0.12	7.35±0.13	7.31±0.12
S1	Fructosamine (µmol/L)	296±5	294±5	295±6	295±6*
S1	FPG (mg/dL)	136±4	131±3	140±6	130±4
S2	HbA1c (%)	7.26±0.12	7.18±0.13	7.09±0.12	7.16±0.13
S2	Fructosamine (µmol/L)	301±6	293±5	288±6*	293±5*
S2	FPG (mg/dL)	141±5	133±4	129±3*	136±4

Supported by: Ministry of Knowledge Economy (MKE), Republic of Korea

## 892

**Zinc supplementation improves glycaemic status in uncontrolled type 2 diabetes mellitus**

H.A. Begum, T. Parveen, T. Ara, M.O. Faruque, Q. Nahar; BIRDEM, Dhaka, Bangladesh.

**Background and aims:** Type 2 Diabetes Mellitus is a chronic, progressive illness that causes considerable morbidity and premature mortality. Zn is an essential mineral that is required for various cellular functions. Its abnormal metabolism is related to certain disorders such as diabetic complications. In Bangladesh there have been very few studies, with contradictory results about the zinc effects on diabetes. The aim of the study to assess the effect of zinc on the glycemic control of type 2 diabetes mellitus subjects.

**Materials and methods:** In this clinical trial 89 diabetic patients (type 2) were divided into two groups, randomly. One of them consumed 20mg/day zinc tablet and the other had no zinc supplement. Body mass index, blood pressure, Fasting blood sugar, 2-h post-prandial glucose, serum triglyceride, serum cholesterol, serum low density lipoproteins, serum high density lipoproteins were checked before and after four weeks.

**Results:** During the four weeks of follow-up the Mean ± SD value of age (yrs), waist circumference (inch), hip circumference (inch), BMI (kg/m<sup>2</sup>), SBP (mmHg) and DBP (mmHg) was 52±7.5, 35.6±5.9, 40±5.6, 26.6±5.3, 129±15, 84±10.5 in zinc supplemented group and the values of without zinc supplemented group were 50±7.6, 35.4±5.6, 39.6±4.7, 24.6±3.6, 131±16.5, 85±10.3 respectively. Mean ± SD value of age, waist circumference, hip circumference, BMI, SBP and DBP did not differed between zinc supplementation group and without zinc supplementation group. The Mean ± SD value of HbA<sub>1c</sub> (%), serum LDL (mg/dl), serum HDL (mg/dl) and serum creatinine (mg/dl) was 9.4±1.95, 128±39, 42±10.5, 1.14±0.4 in zinc supplemented group and the values of without zinc supplemented group were 8.22±1.3, 130±32.1, 47.4±9.9, 1.04±0.5 respectively. Serum lipid profile was similar between zinc supplement and without zinc supplement group. Both the fasting (0 day, 12.7±3.2 vs 30<sup>th</sup> day, 10±2.8) and after breakfast (0 day, 16±4.2 vs 30<sup>th</sup> day, 10.2±6) serum glucose in subjects with normal serum triglyceride (TG) levels (<150 mg/dl) were significantly (P<0.01) decreased in zinc supplemented group after four weeks supplementation whereas only serum glucose after breakfast was decreased significantly in subjects with abnormal TG values (> 150 mg/dl). No decreasing tendency was found in subjects without Zinc supplementation. Both the fasting and after breakfast serum glucose levels in subjects with both normal (serum Chol <200 mg/dl, Fasting 13.2±3 vs 10.4±2.7; ABF 17±4 vs 9.4±6.4) and hyper cholesterol group (serum Chol >200 mg/dl Fasting 11.8±3 vs 9.4±2.5; ABF 16±4.3 vs 10±5.4) were significantly decreased in Zinc supplemented group after four weeks supplementation whereas no decreasing tendency was found in subjects without Zinc supplementation. Both the fasting and after breakfast serum glucose levels in subjects with both normal (Fasting 13.4±3 vs 10.4±2.4; ABF 17.7±4.4 vs 12.5±3) and higher waist circumference levels were significantly decreased in Zinc supplemented group (Fasting 12.5 ±3 vs 10.0 ±3.0; ABF 16.2±4.0 vs 8.3±4.0) after four weeks supplementation whereas no decreasing tendency was found in subjects without Zinc supplementation.

**Conclusion:** Our result indicate that Zinc supplementation is significant for improving fasting and post-prandial glycemic control in type 2 diabetes mellitus patients with normal serum TG level.

Supported by: BIRDEM

## 893

**Low consumption of wholegrain products predicts a higher incidence of prediabetes and type 2 diabetes**

A. Björklund, T. Wirstrom, A. Hilding, H.F. Gu, C.-G. Östenson; Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** Low intake of wholegrain and fiber have been associated with the development of type 2 diabetes (T2DM). However, data from prospective studies including effects on prediabetic states are scarce. Furthermore an interaction with the TCF7L2 gene has been suggested but not confirmed in new studies. We investigated in a prospective population-based study whether low intake of wholegrain predicts evolution of insulin resistance, pre-diabetes and T2DM. We also tested for modulation by polymorphisms of the TCF7L2 gene.

**Materials and methods:** We examined the 8–10-year incidence of pre-diabetes and T2DM in relation to intake of wholegrain and fiber at baseline among 3180 women and 2297 men aged 35–56 y participating in the Stockholm Diabetes Prevention Program. We used multiple logistic regression analysis to calculate Odds ratios (OR) together with 95% confidence intervals.

**Results:** The risk to deteriorate in glucose tolerance (to pre-diabetes, i.e. IGT and/or IFG, or to T2DM) was increased by 80 % in men with a low intake of wholegrain (OR=1.8, 1.4–2.3). The association remained after adjustment for age, family history of diabetes, BMI, physical activity, smoking, education and blood pressure. Similar, though weaker associations were seen for total intake of fiber. Low intake of wholegrain was correlated with a parameter of insulin resistance (HOMA-IR). A protective effect of wholegrain intake was undetectable in men harbouring diabetogenic polymorphisms of the TCF7L2 gene. In women no significant associations were found between intakes of wholegrain and the risk of pre-diabetes or T2DM.

**Conclusion:** In men low intake of wholegrain predicts development not only of T2DM but also pre-diabetes by mechanisms likely tied to effects on insulin sensitivity. Further, we confirm effect modifications by TCF7L2 genetic polymorphisms.

Supported by: Stockholm County Council, Swedish Research Council, Swedish Diabetes Ass.

## 894

### A novel hydrophilic derivative of probucol ameliorates glucose tolerance and insulin sensitivity independently of the canonical potency of probucol

L. Miyamoto<sup>1,2</sup>, M. Kono<sup>1</sup>, T. Nakagawa<sup>1</sup>, H. Hattori<sup>2</sup>, K. Ishizawa<sup>1</sup>, H. Nemoto<sup>2</sup>, K. Tsuchiya<sup>1</sup>;

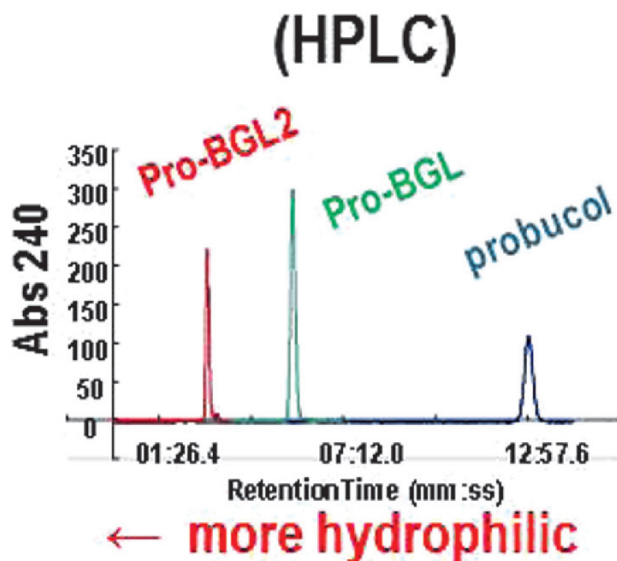
<sup>1</sup>Medical Pharmacology, Inst. of Health Biosciences, <sup>2</sup>Pharmaceutical Chemistry, The University of Tokushima Graduate School, Tokushima, Japan.

**Background and aims:** Probuco is a traditional anti-hyperlipidemic agent harboring marked antioxidant activity. While probucon is extremely hydrophobic, its succinic acid ester, succinobucol, was reported as a water-soluble derivative and was in clinical trials. Succinobucol was shown to be more potent in lipid-lowering, anti-inflammatory and anti-atherogenic effects than probucon in animal models. In addition, succinobucol reduced HbA1c in diabetic patients. Although the clinical trials finally ended in failure, it appears a promising strategy for drug discovery to modulate water-solubility of established active compounds. Meanwhile, we have reported chemical modification of lipophilic agents with symmetrically branched glycerol oligomers (BGL) as a suitable approach to intensify hydrophilicity. Thus we designed and synthesized a novel highly hydrophilic derivative of probucon, Probucon-(glutaric branched-triglycerol)<sub>2</sub> (ProBGL2) and evaluated its effects on glucose and lipid metabolism in high fat diet (HFD)-fed mice.

**Materials and methods:** Lipophilic-hydrophilic balance was assessed by determining octanol-water partition coefficient values (Po/w) using a reversed phase HPLC. Anti-lipid peroxidation activity was estimated by TBARS assay in vitro. 1 g/kg/day of probucon or ProBGL2 were orally administered for a week to male ddY mice at the age of 24 wk fed HFD for 10 wk, and then ipGTT was performed. Insulin level and lipid parameters in the plasma were also determined.

**Results:** Retention time of ProBGL2 was less than one tenth of probucon, suggesting striking improvement of hydrophilicity. In accordance, the Po/w of ProBGL2 was more than 100-times less than that of probucon. However, TBARS assay revealed ProBGL2 possesses far less antioxidant activities against lipid peroxidation than probucon. In HFD-fed mice, while body weight and food intake were not affected, glucose tolerance was much improved by administration of probucon or ProBGL2 (AUC; -50%). Fasting plasma insulin levels and HOMA-IR in the both groups were 70% decreased, suggesting marked amelioration of insulin resistance. However, ProBGL2 did not change plasma total cholesterol concentration though probucon lowered it more than 50%. Furthermore, Plasma triglyceride and NEFA levels were not altered.

**Conclusion:** Our data demonstrate that probucon and its hydrophilic derivative, ProBGL2 ameliorate glucose intolerance and insulin resistance in HFD-fed mice and those are independent of the canonical potency of probucon such as lipid-lowering or antioxidant activities. The effects of ProBGL2 on glucose tolerance and insulin sensitivity were stoichiometrically more than twice as potent as those of probucon. ProBGL2 is suggested to be a novel potent anti-diabetic agent.



Supported by: Japan Science and Technology Agency

## PS 072 Diabetes in childhood

## 895

### Association between growth in early infancy and the development of islet autoimmunity and type 1 diabetes

M. Pflueger<sup>1</sup>, E. Thiering<sup>2</sup>, A. Knopff<sup>3</sup>, J. Stock<sup>3</sup>, J. Heinrich<sup>2</sup>, A.-G. Ziegler<sup>1,3</sup>;

<sup>1</sup>Institute of Diabetes Research, Helmholtz Zentrum München, Klinikum rechts der Isar, Technische Universität München, Neuherberg/Munich,

<sup>2</sup>Institute for Epidemiology I, Helmholtz Zentrum München, Neuherberg/Munich, <sup>3</sup>Forschungsgruppe Diabetes e.V., Neuherberg, Germany.

**Background and aims:** Recent longitudinal epidemiologic studies have suggested that weight gain and weight gain velocity during infancy might have a priming effect on adiposity, blood pressure and asthma later in life. A recent longitudinal study showed an association between weight gain during infancy and onset of asthma in childhood. With a longitudinal model, peak height and weight velocities during the first 2 years of life were analysed. The aim of this study was to evaluate the associations between growth velocity in infancy and the risk of islet autoimmunity and type 1 diabetes.

**Materials and methods:** Data on weight and height were collected at the ages 1, 2, 3, 6, 12 and 24 months from 958 children participating in the BABYDIAB and BABYDIET study, including 106 children who developed persistent islet autoantibodies. All children had a first-degree relative with type 1 diabetes and were prospectively followed from birth for the development of islet autoantibodies defined as one or more autoantibodies to insulin, GAD or IA-2 in two or more blood samples. Individual weight and height growth curves through age 24 months were calculated according to the nonlinear Reed1 model. Peak height velocity (PHV) and peak weight velocity (PWV) were calculated and are defined as the maximum of the first derivatives of the height and weight growths curves, respectively. Age and BMI at adiposity peak and adiposity rebound were derived from cubic mixed models with random effects for two age groups. All models were adjusted for gender.

**Results:** The adiposity peak was significantly associated with the development of islet autoantibodies. There was a linear correlation between the adiposity peak and the risk for the development of one or more islet autoantibodies (one islet autoantibody: HR 0.6 [95% CI 0.4-0.9]; p=0.018 and multiple islet autoantibodies: HR 0.4 [95% CI 0.2-0.8] per 2SD increase; p=0.006). Children's adiposity peak were classified in quartiles which showed that children with a adiposity peak in the first quartile have a significant higher risk for the development of islet autoantibodies compared to children in the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> quartile (at age 10 years: 1<sup>st</sup> quartile 8.1% vs 2<sup>nd</sup> and 3<sup>rd</sup> quartile 4.4% vs 4<sup>th</sup> quartile 2.9%, p=0.017). The adiposity peak was not associated with the progression to type 1 diabetes. PHV, PWV and adiposity rebound in childhood were not associated with the development of islet autoantibodies, independently from timing and level.

**Conclusion:** These data suggest that an early relative weight gain in the first years of life increase the risk for the development of islet autoimmunity in childhood. The exact mechanisms involved are not yet clear and further examinations are required.

Clinical Trial Registration Number: NCT01115621

Supported by: FKZ 01GI0805-07, DFG ZI-310/14-1 to 14-4, JDRF; no 1-2006-665

## 896

### Wrist circumference positively correlates with systolic and diastolic blood pressure in overweight/obese children and adolescents

M. Capizzi<sup>1</sup>, G. Campagna<sup>1</sup>, G. Leto<sup>1</sup>, S. Zampetti<sup>1</sup>, M. Spoletini<sup>1</sup>, C. Venditti<sup>1</sup>, M. Mastantuono<sup>2</sup>, A. Vania<sup>3</sup>, R. Buzzetti<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine and Medical Specialities, Sapienza,

<sup>2</sup>Department of Radiology, Sapienza, <sup>3</sup>Department of Pediatrics, Sapienza, University of Rome, Italy.

**Background and aims:** Cardiovascular diseases are the leading cause of death in Western countries. One of the most important cardiovascular risk factors according to many pathophysiological models is insulin resistance. The wrist circumference, in particular its bone component, has been recently demonstrated to be a clinical marker for insulin-resistance in overweight/obese children, configuring the possibility that this parameter could be taken into account in the measurement of cardiovascular risk. Another risk factor for the developing of cardiovascular disease is blood pressure. Recent studies have shown an acceleration of skeletal growth in children in case of primary

hypertension. In the light of these findings, the aim of the present study was to evaluate the presence of a correlation between wrist circumference and blood pressure in overweight/obese children and adolescents.

**Materials and methods:** N=140 overweight/obese children and adolescents (mean age  $9.8 \pm 2.8$  years), were consecutively recruited for the evaluation of blood pressure, wrist circumference, weight and waist circumference. In all subjects blood samples were taken in order to evaluate insulin and blood glucose and to calculate the HOMA-IR (Homeostasis Model Assessment of Insulin Resistance). The analysis was performed by General Linear Model with the SAS software v.9.2.

**Results:** The wrist circumference was significantly and positively associated with systolic and diastolic blood pressure ( $p < 0.05$  and  $p < 0.0001$  respectively), explaining in the model 10% of the systolic pressure variability and 25% of the diastolic pressure variability.

**Conclusion:** The circumference of the wrist in overweight/obese children and adolescents is correlated with systolic and diastolic blood pressure, confirming that this bone anthropometric marker could be of potential interest for the prediction of cardiovascular risk.

## 897

### Prevalence of type 1 diabetes mellitus in children and adolescents in Germany

J. Rosenbauer<sup>1</sup>, C. Bächle<sup>1</sup>, A. Stahl<sup>1</sup>, K. Castillo<sup>1</sup>, T. Meissner<sup>2</sup>, R.W. Holl<sup>3</sup>, G. Giani<sup>1</sup>, for German Pediatric Surveillance Unit, DPV Initiative, German Competence Network Diabetes mellitus;

<sup>1</sup>Institute for Biometry and Epidemiology, German Diabetes Centre, Düsseldorf, <sup>2</sup>University Children's Hospital Düsseldorf, <sup>3</sup>Institute for Epidemiology and Medical Biometry, Ulm University, Germany.

**Background and aims:** The incidence of type 1 diabetes in children and adolescents has been shown to increase in Germany. Up to date prevalence estimates of type 1 diabetes are a basic requirement for allocation of health care resources for type 1 diabetes care. Aim of the present study was to provide updated estimates on the prevalence of type 1 diabetes in children and adolescents in Germany based on the diabetes incidence register of North Rhine-Westphalia, the most populous German federal state.

**Materials and methods:** Since 1996 newly diagnosed cases of type 1 diabetes have been ascertained with the North Rhine-Westphalian diabetes register by means of three data sources, the prospective hospital-based active surveillance system ESPED, annual inquiries among paediatric, internal and general medical practices, and the computer-based documentation system DPV. The DPV initiative was founded for quality control and scientific research in paediatric diabetes care. Completeness of ascertainment was estimated by the capture-recapture-method. Point and interval estimates (95%-CI) of prevalences (per 100,000 persons) were based on Poisson distribution. Age- and sex-standardized prevalences were estimated by the direct method using equal weights. Poisson regression analysis was applied to assess the effects of age and gender.

**Results:** On 31.12.2010, 4,069 children and adolescents with type 1 diabetes 0-14 years of age and 7,348 children and adolescents 0-19 years of age were registered in North-Rhine Westphalia. The completeness of ascertainment in the both age groups was estimated to be 98.8% (98.4-99.2%) and 98.1% (97.7-98.5%), respectively. The age- and sex-standardized prevalence estimates was 161.8 (156.9-166.8) in the 0-14-year-olds and 207.5 (202.8-212.3) in the 0-19 year-olds, respectively. The prevalences among boys and girls in the age group 0-14 years were quite similar 161.4 (154.4-168.3) vs. 162.3 (155.2-169.5),  $p=0.835$ . In the age group 0-19 years, the prevalence among males was significantly higher than among females (214.9 (208.1-221.6) vs. 199.8 (193.1-199.8),  $p=0.002$ ), owing to a significant male preponderance in the age group 15-19 years (356.2 (340.1-373.0) vs. 298.8 (283.7-314.6),  $p=0.002$ ). The prevalences in the age groups 0-4, 5-9, 10-14, and 15-19 years were 34.8 (30.6-39.1), 152.6 (144.0-161.1), 279.4 (268.6-290.2), and 328.3 (317.0-339.5) ( $p < 0.001$ ), respectively.

**Conclusion:** The North Rhine-Westphalian diabetes register provides valid estimates of type 1 diabetes prevalence in childhood and adolescence in Germany. At present, about 16 and 21 out of 10,000 children and adolescents in the age group 0-14 and 0-19 years, respectively, have type 1 diabetes. Based on the current estimates, overall there are approximately 17,200-18,300 0-14-year-olds and 30,600-32,100 0-19-year-olds with type 1 diabetes in Germany, respectively.

*Supported by: Competence Network Diabetes mellitus funded by BMBF (01GI0802, 01GI0859)*

## 898

### Prevalence of increased liver enzymes in 15466 children and adolescents with type 1 diabetes mellitus and type 2 diabetes mellitus in the DPV cohort

M.J. Fritsch<sup>1</sup>, E. Schober<sup>1</sup>, J. Grulich-Henn<sup>2</sup>, T. Meissner<sup>3</sup>, T. Kapellen<sup>4</sup>, D. Dunstheimer<sup>5</sup>, P. Beyer<sup>6</sup>, C. Vogel<sup>7</sup>, E. Molz<sup>8</sup>, R.W. Holl<sup>8</sup>, German Competence Network Diabetes mellitus and the DPV initiative; <sup>1</sup>Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Austria, <sup>2</sup>Center for child and adolescent medicine, Heidelberg University Hospital, Germany, <sup>3</sup>Department of General Pediatrics and Neonatology, University's Children Hospital of Duesseldorf, Germany, <sup>4</sup>Department for Women and Child Health, University of Leipzig, Germany, <sup>5</sup>Clinic for Children and Juveniles, University of Augsburg, Germany, <sup>6</sup>Department of Pediatrics and Adolescent Medicine, Evangelic Hospital Oberhausen, Germany, <sup>7</sup>Clinic for Paediatrics and Adolescent Medicine, Klinikum Chemnitz, Germany, <sup>8</sup>Institute for Epidemiology and medical Biometry, University of Ulm, Germany.

**Background and aims:** A persistent elevation of liver enzymes may be the first indicator of the underlying condition of non alcoholic fatty liver disease (NAFLD). Data about the prevalence of NAFLD in childhood T1DM is rare. The aim of the study was to compare the prevalence of elevated aminotransferases (TAs) in a large cohort of children and adolescents with T1DM and T2DM and investigate possible relations to cardiovascular risk factors.

**Material and methods:** Data of 15201 patients (47.1% female) with T1DM and 265 patients (60.4% female) with T2DM from the German-Austrian DPV (Diabetes Patienten Verlaufsbeobachtung) were included into the analyses. The observation period was from January 1995 to September 2011. Inclusion criteria were an age  $< 20$  years and at least one measurement of TA in the most recent year of treatment. Elevated TAs were defined as ALT and/or AST  $> 50$  U/l. Patients with celiac disease were excluded from the analysis.

**Results:** Patients' median age was 14.5 (10.8-17.2) years vs. 15.9 (14.1-17.3) years with a median HbA1c of 7.9% (7.1-9.1) vs. 6.8% (5.9-8.5) in patients with T1DM and T2DM respectively. In patients with T1DM, 95.4% ( $n=14500$ ) had normal TAs whilst they were increased once in 4.0% ( $n=605$ ) and twice or more in 0.6% ( $n=96$ ). In patients with T2DM, 54% ( $n=143$ ) had normal TAs, which were increased once in 34.3% ( $n=91$ ) and twice or more in 11.7% ( $n=31$ ). In patients with T1DM increased TAs were associated with higher age ( $p < 0.001$ ), HbA1c ( $p < 0.001$ ) and insulin dose ( $p=0.01$ ), shorter stature ( $p < 0.0001$ ), dyslipidaemia ( $p < 0.001$ ) and arterial hypertension ( $p=0.048$ ). No significant association was found between elevated TAs and sex and weight in T1DM. In patients with T2DM, increased TAs were associated with higher age ( $p=0.04$ ) and weight ( $p=0.01$ ), male sex ( $p=0.02$ ), dyslipidaemia ( $p=0.02$ ) and arterial hypertension ( $p < 0.01$ ).

**Conclusion:** In our cohort, the prevalence of elevated liver enzymes was 10 fold higher in children with T2DM compared to children with T1DM. Age and cardiovascular risk factors like dyslipidaemia and arterial hypertension have increased the risk for NAFLD in both, T1DM and T2DM. Insufficient glycemic control and higher daily insulin dose were associated with higher risk for NAFLD in patients with T1DM only, whereas weight and male sex were positively related with NAFLD only in patients with T2DM.

## 899

### The influence of vitamin D analogue on dendritic cells subpopulations in children with type 1 diabetes mellitus

R. Piekarski<sup>1</sup>, L. Szewczyk<sup>1</sup>, J. Tabarkiewicz<sup>2</sup>, J. Roliński<sup>2</sup>;

<sup>1</sup>Pediatric Endocrinology and Diabetology, Medical University in Lublin,

<sup>2</sup>Clinical Immunology, Medical University in Lublin, Poland.

**Background and aims:** In type 1 diabetes dendritic cells (DC) plays an important role in the initiation and modulation of immune response against antigens of pancreatic islet  $\beta$  cells. The aim was evaluation of circulating myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) in children with type 1 diabetes before and after application of the vitamin D3 analog (Alphacalcidol) in both groups and compared to a group of healthy children.

**Materials and methods:** The study group comprised 50 children, mean age  $10 \pm 5$  years, with newly diagnosed type 1 DM who were randomly enrolled into two groups (treated with vitamin D3 analog or not) and subjected to annual follow-up. In all children were assessed twice the level of 25 (OH) D3, C-peptide and anti-GAD and anti-IA2 antibodies and cell subpopulations were examined using flow cytometry. The reference group consisted of 10 healthy



children. We analyzed the percentage of immature myeloid DC BDCA-1+ / CD19- and plasmacytoid BDCA-2+ / CD123+.

**Results:** In 72% of children who received Alphacalcidol and in 50% without Alphacalcidol, an increase or maintain the value of C-peptide during the annual monitoring as compared with baseline values was observed. In the blood of children with type 1 diabetes not receiving Alphacalcidol the average percentage of myeloid DCs was 0.79% and was significantly higher ( $p < 0.05$ ) than in healthy children (0.26%). However, there were no differences in the percentage of BDCA1 + cells between the group receiving Alphacalcidol and a control group. The percentage of plasmacytoid cells did not differ significantly between groups. The dynamics of changes in the percentage of DC subsets in relation to baseline (newly diagnosed diabetes) was analyzed.

**Conclusion:** The demonstrated differences of the analyzed parameters and the population of immune system cells encouraging more detailed analysis of the observed dependence, because they seem to indicate certain positive elements of used vitamin D3 analog in children with type 1 diabetes.

Supported by: MNiSzW NN407 310033

## 900

### The relationship between the quality of daily glycaemic self-monitoring and HbA<sub>1c</sub> in type 1 diabetes mellitus children from Romania, during 2008–2011

V. Serban<sup>1,2</sup>, B. Timar<sup>1,3</sup>, L. Barna<sup>2</sup>, A. Lacatusu<sup>2</sup>, A. Sima<sup>1,3</sup>, M. Rosu<sup>1,3</sup>,  
<sup>1</sup>"Victor Babes" University of Medicine and Pharmacy, Timisoara, <sup>2</sup>"Cristian Serban" Medical Center, Buzias, <sup>3</sup>County Clinical Emergency Hospital, Timisoara, Romania.

**Background and aims:** It has been worldwide observed that obtaining an adequate HbA<sub>1c</sub> value corresponding to an optimal glycemic control in childhood type 1 diabetes mellitus is a difficult challenge. This aspect depends on morphophysiological changes due to age and on many other external factors, including poor medical education and the limited access to an optimal glycaemic self-monitoring. The main aim of our study was to prove the tight inverse correlation between the number of daily self-monitoring tests and HbA<sub>1c</sub> value, which, as we know, is an effective indicator of glycemic control.

**Materials and methods:** We analyzed 1396 admissions of 774 unique patients aged between 1 and 18 years, in our Medical Center between 2008 and 2011. From these patients, 763 were following basal bolus insulin therapy and 11 were on insulin pumps. Patients were grouped according to the number of daily self-monitoring tests prior to admission (stable number for at least 3 months before admission). We analyzed the entire study group characteristics and also, individually for the following age groups: 1 to 6 years old, 7 to 10, 11 to 14 and 15 to 18 years old. The HbA<sub>1c</sub> values were measured for every individual in the second day of admission (using immunoturbidimetric assay method, reference values: 4.6 to 5.9%). Statistics compared the difference between all possible pairs studied (from 0 to 4 self-monitoring tests daily), using ANOVA test for the entire variance and unpaired two-tailed t test for each individual pair.

**Results:** We observed an extremely significant decrease of mean HbA<sub>1c</sub>, with 2.94 % (95%CI from 2.39% to 3.50%,  $p < 0.001$ ) for the entire study group with the increase in the number of daily self-monitoring tests from 0 (mean HbA<sub>1c</sub>: 9.82%) to 4 per day (mean HbA<sub>1c</sub>: 7.98%). Also, a decrease of HbA<sub>1c</sub> was found for every individual age group studied, as following: in 1 to 6 years old an average decrease of 3.15% (95%CI from 1.29% to 5.00%,  $p < 0.001$ ); in 7 to 10 years old group an average of 2.98% (95%CI from 1.97% to 4.00%,  $p < 0.001$ ); in 11 to 14 years old an average decrease of 3.10% (95%CI from 1.97% to 4.23%,  $p < 0.001$ ) and for 15 to 18 years old a decrease of 2.35% (95%CI from 1.31% to 3.40%,  $p < 0.001$ ). For the entire study group, extremely significant reductions were observed between all possible pairs of groups with a daily number of 0, 1, 2, 3 or 4 self-monitoring tests, excluding here the pair with no testing and one daily test, where the differences were only very significant, and the pair containing one and two daily tests between which the differences were not significant.

**Conclusion:** In our patients (children aged between 1 and 18 years) we were able to prove the existence of a strong, inverse correlation between the daily number of glycemic tests and the HbA<sub>1c</sub> values. Excepting one pair of groups (composed by one vs. two daily tests), there were very and extremely significant improvements in the HbA<sub>1c</sub> values for each increase with one test per day. Best results are corresponding to at least four daily tests and the lowest number of still efficient tests is two per day.

## 901

### Insulin sensitivity since the preschool to the school age in severely obese children

M. Manco, M.R. Spreghini, R. Luciano, C. Pensini, R.S. Wietrzycowska, C. Rustico, G.S. Morino;  
 Scientific Directorate, Bambino Gesù Hospital, Rome, Italy.

**Background and aims:** It has been widely described that insulin sensitivity decreases at the puberty, but no information is provided on its earlier time-course. The present study aimed at describing time-course of insulin sensitivity in severely obese children at the transition from the preschool age to the pre-pubertal period.

**Materials and methods:** Retrospective study of 47 severely obese (BMI  $\geq 99^{\text{th}}$  percentile) evaluated between ages 2–6 years and re-evaluated before age 8 years with estimation of glucose tolerance, insulin sensitivity, secretion and glucose disposition index estimated during the oral glucose tolerance test by means of the Whole Body Insulin Sensitivity Index (WBISI), the Insulino-genic Index (IGI); and the Insulin Secretion-Sensitivity Index 2 (ISSI2). Normal weight age- and sex- matched patients undergoing minor surgery served as controls for fasting insulin resistance (Homeostasis Model Assessment of Insulin Resistance, HOMA-IR), lipid profile and liver function tests.

**Results:** Obese patients and controls showed significant different HOMA-IR and lipid profile both at baseline and follow-up ( $p < 0.0001$  for all the comparisons). At follow-up (mean interval  $2.22 \pm 0.66$  years), obese patients showed significant decrease in WBISI ( $p < 0.0001$ ), fasting ( $p = 0.005$ ) and 2 h glucose (2HG,  $p = 0.001$ ). Importantly, 1 child presented at baseline and 4 at follow-up with 2HG between 140 and 199 mg/dl. At stepwise regression models, predictors of WBISI and 2HG at follow-up were changes in BMI z-score ( $R^2 = 0.371$ ;  $p = 0.003$ ;  $\beta = -0.609$ ), and ISS-2 ( $R^2 = 0.411$ ;  $p = 0.001$ ;  $\beta = -0.641$ ), respectively.

**Conclusion:** In obese preschoolers, the decline of insulin sensitivity seems to occur even before the pubertal transition.

Supported by: RF-OPG-20081142374; RC 201102T002598; RC 201102R002566

## 902

### Interim safety results from a European observational cohort study of children ages 6–12 with type 1 diabetes treated with insulin glulisine (OCAPI study)

M. Konstantinova<sup>1</sup>, V. Loizeau<sup>2</sup>, V. Pilonet<sup>3</sup>, A. Cali<sup>3</sup>, T. Danne<sup>4</sup>;  
<sup>1</sup>University Pediatric Hospital, Sofia, Bulgaria, <sup>2</sup>Lincoln, Boulogne-Billancourt, France, <sup>3</sup>Sanofi, Paris, France, <sup>4</sup>"Auf der Bult" Diabetes Centre for Children, Hannover, Germany.

**Background and aims:** Children with type 1 diabetes (T1DM), particularly younger children, are at risk of clinically significant hypoglycaemia especially when low HbA<sub>1c</sub> levels, missed meals, and increased activity are involved. To enhance our knowledge of the safety of insulin glulisine, we observed children aged 6–12 with T1DM who were given insulin glulisine over a 6-month period in a real-life clinical practice setting, with special focus on those aged 6–8.

**Materials and methods:** OCAPI is an observational, prospective cohort study with 6-month follow-up; interim results were conducted when half of the subjects completed the study. The population comprised children aged 6–12 with T1DM on a stable insulin regimen for  $\geq 3$  months, for which physicians prescribed insulin glulisine. Visits were conducted according to clinical practice; data were recorded after 3 and 6 months of treatment. This analysis included incidence of severe hypoglycaemia in children aged 6–12 (primary objective) with particular focus on the subgroup aged 6–8, as well as symptomatic hypoglycaemia, injection site/systemic hypersensitivity reaction, and medication error in all age groups (secondary objective).

**Results:** Of the 70 patients considered for analysis, 25 were aged 6–8. At inclusion, median duration of insulin treatment was 1.0 y (Q1–Q3: 1.0–3.0) and 2.0 y (Q1–Q3: 1.0–3.0) for the 6–8 and 9–12 groups, respectively; mean HbA<sub>1c</sub> was  $8.0 \pm 1.2\%$  and  $8.3 \pm 1.4\%$ . During the study, number of daily basal insulin injections did not change. Symptomatic documented hypoglycaemia were reported in 56 (81.2%) patients, with similar results among the 6–8 and 9–12 groups. For the on-treatment period, the 6–8 group had higher median number of symptomatic documented hypoglycaemia (18.5) than the 9–12 group (12.0). Incidence rate was numerically higher in the 6–8 group vs the 9–12 group (7573 vs 6676 per 100 patients/y). Overall, incidence of severe hypoglycaemia was 3.0 events per 100 patients/y (Table). Only 1 patient, age 10 y, experienced severe hypoglycaemia, 48 days after insulin glulisine initiation. Injection site reactions ( $n = 16$ ) and medication errors ( $n = 1$ ) occurred only

in the 9–12 group; no patients presented with systemic hypersensitivity reactions. No change in  $HbA_{1c}$  ( $-0.1 \pm 1.5\%$ ) was observed after inclusion.

**Conclusion:** The study reveals that hypoglycaemic events are frequent and occur predominantly in the younger age group. Only 1 severe case of hypoglycaemia was observed, which is encouraging. The incidence of severe hypoglycaemia in children aged 6–12 y treated with insulin glulisine was lower than ranges previously reported in this population (9.4–73 events per 100 patients/y). These data confirm that insulin glulisine is safe in this young population.

Incidence rate of severe hypoglycaemia per 100 patients/year.

	Age Groups		Total
	6 to 8 years old (n=25)	9 to 12 years old (n=45)	(N=70)
Incidence Rate (per 100 patients/year)	0	4.4	3.0
95% CI (Poisson distribution approximation)		[0.1; 24.8]	[0.1; 16.6]

Supported by: Sanofi

## 903

### The quality of glycaemic control in type 1 diabetes mellitus children from Romania, between 1998–2010

B. Timar<sup>1,2</sup>, V. Serban<sup>2,3</sup>, L. Barna<sup>3</sup>, A. Lacatusu<sup>3</sup>, F. Fiera<sup>3</sup>, A. Sima<sup>1,2</sup>, M. Rosu<sup>1,2</sup>, A. Vlad<sup>1,2</sup>,

<sup>1</sup>County Clinical Emergency Hospital, Timisoara, <sup>2</sup>“Victor Babes” University of Medicine and Pharmacy, Timisoara, <sup>3</sup>“Cristian Serban” Medical Center, Buzias, Romania.

**Background and aims:** Reaching an optimal glycaemic control (according current standards), right after diagnosis and maintaining it for the rest of its life, is the main goal of treatment in children with type 1 Diabetes Mellitus (DM) as it is the most important prevention factor regarding chronic complications and it extends the life expectancy. Unfortunately, in each and every country, this target is extremely difficult to obtain, the quality of glycaemic control must be continuously monitored in order to act promptly where needed. Our study main aim is to evaluate the evolution of  $HbA_{1c}$  values in time, in a representative number of children with type 1 DM from Romania, which we are regarding to be an adequate long term indicator of glycaemic control, in order to reach the information we needed to further correct, where is needed, the existing layout.

**Materials and methods:** To evaluate the long term  $HbA_{1c}$  values behavior (between 1998–2010) in children with type 1 DM, from the entire Romanian area, we enrolled 1718 individuals, aged between 1 and 18 years, all of them being at their first admission in our medical center, the only hospital in country specialized in evaluation, treatment and education of children with type 1 Diabetes Mellitus. The  $HbA_{1c}$  values were measured for each individual in the second day of admission, which we consider to reflect the quality of diseases management obtained in outpatient conditions. Using a retrospective study we analyzed the significance of  $HbA_{1c}$  values variation in the entire 1998–2010 period and the variation between the following periods: 1998–2000; 2001–2003; 2004–2007 and 2008–2010.

**Results:** The decrease of average  $HbA_{1c}$  value from 11.17% (95%CI: 10.71%–11.58%) in 1998 to 8.36% (CI: 8.02%–8.70%) in 2010 is extremely significant, both clinically and statistically; however this value is far from an optimal glycaemic control. The period between 1998 (mean  $HbA_{1c}$  11.17%) and 2000 (mean  $HbA_{1c}$  8.64%) is marked by a significantly yearly decrease of average  $HbA_{1c}$ , followed by a period without significantly variance (2001–2003). The 2004–2007 interval is characterized by an elevated mean  $HbA_{1c}$  compared with the previous period, but with no significant variance within its contained years. Finally, we observed a new extremely significant lowering of  $HbA_{1c}$  mean from 2007 to 2008, followed by a maintaining of mean values until 2010 (8.36%, CI: 8.02%–8.70%).

**Conclusion:** The mean  $HbA_{1c}$  values between 1998 and 2010 for a significant sample for Romania of type 1 DM children decreased extremely significant (with 2.81  $HbA_{1c}$  points,  $p < 0.001$ ). This values reached two minimums, for 2001–2003 (mean  $HbA_{1c}$ : 8.56%, CI 8.15%–8.96%,  $n=356$ ) and 2008–2010 (mean  $HbA_{1c}$ : 8.49%, CI 8.32%–8.66%,  $n=540$ ). Considering that this two minimum values, separated in time, are very alike in spite of a meanwhile enhancement in insulin therapy, of using insulin analog in most of the cases and despite the expanded access to self-monitoring (still dissatisfactory,

though) allows us to suppose that a new improvement of the glycaemic control cannot be reached without complementary methods such as wide access to optimal self-monitoring, a solid education performed by trained teachers and improvement of the economic status of children families.

## 904

### Natural Killer T cells (NKT) in children with new onset type 1 diabetes - preliminary report

L. Szewczyk<sup>1</sup>, R. Piekarski<sup>1</sup>, A. Bojarska-Junak<sup>2</sup>, J. Roliński<sup>2</sup>,

<sup>1</sup>Pediatric Endocrinology and Diabetology, <sup>2</sup>Clinical Immunology, Medical University in Lublin, Poland.

**Background and aims:** Type 1 diabetes is a disease of autoimmune pathogenesis in which different populations of immune cells plays an important role in the initiation and modulation of immune response against antigens of pancreatic islet  $\beta$  cells. NKT cells (natural killer T cells) are a heterogeneous group of cells that plays an important role in the immune response affecting the activity including dendritic cells, T cells, B cells, Tregs through several secreted cytokines. It seems that by their production of Th2 cytokines, NKT may have a protective role against autoimmune diseases, including type 1 diabetes. The aim of our study was to evaluate circulating natural killer T cells (NKT) in children with new onset type 1 diabetes and comparison to a group of healthy children.

**Materials and methods:** The study group comprised 32 children, mean age  $10 \pm 5$  years, with newly diagnosed type 1 diabetes. In all children were assessed C-peptide and anti-GAD and anti-IA2 antibodies to confirm autoimmune pathogenesis of disease and cell subpopulations were examined using flow cytometry. The reference group consisted of 20 healthy children. We analyzed the percentage of NKT3/161+, NKT/4+ cells.

**Results:** In the blood of children with type 1 diabetes the average percentage of NKT was  $51.11 \pm 19.60\%$  and was significantly lower ( $p < 0.05$ ) than in healthy children  $76.56 \pm 16.7\%$ .

**Conclusion:** The demonstrated differences of the analyzed population of immune cells encouraging more detailed analysis of the observed dependence, because they seem to indicate certain disturbances in investigated population of cells in children with type 1 diabetes.

Supported by: MNiSzW NN407 160740

## 905

### Study of the outcomes of application of ISPAD versus ADA guidelines of diabetic ketoacidosis in type 1 diabetic children

M.M.F. El Hefnawy, N. Own;

Pediatric Endocrinology, National Institute of Diabetes & Endocrinology, Cairo Egypt

**Background and aims:** Diabetic ketoacidosis (DKA), is the most common and serious complication of type 1 diabetes. There are 2 main guidelines for management of DKA in children under 20 years old. These 2 guidelines are: American Diabetes Association guidelines (ADA), and the International Society of Pediatric and Adolescent Association (ISPAD)/ International Society of Diabetes (IDF) one. The aim of this study was to study the outcomes of application of every one of these 2 guidelines.

**Materials and methods:** Each protocol has been applied on 100 type 1 diabetic child with DKA that had been diagnosed according WHO guidelines. The follow up of both groups were done by doing evaluation of conscious level hourly by Glasgow coma score estimation, in addition to do the following laboratory analysis: ph, serum Bicarbonatr, serum potassium, and all other electrolyte and gas levels, till recovery from the DKA.

**Results:** The results of this study showed that the protocol of fluid therapy according to ISPAD gave better results than the ADA protocol. The insulin therapy outcomes of both of them gave same results, although it was better to delay the insulin therapy for 1 hour after giving the fluid therapy. As regarding the potassium, it was much more significantly better to give the potassium according the ISPAD/IDF protocol as there was significantly less hypokalemia and it was much more better not to stop giving insulin except if potassium level  $< 2.5$  mmol/l.

**Conclusion:** The results of this study directed us to follow the ISPAD/IDF protocol for management of type diabetic children less than 20 years with DKA, starting with fluid therapy. After 1 hour, you can start insulin and potassium therapy and to advise to stop insulin only if its level decreased to less than 2.5 mmol/l.

## PS 073 New insulins I

### 906

**Development of ultra-rapid-acting prandial insulin analogues requires chelation of zinc ions charge masking to increase the rate of subcutaneous absorption**

R. Pohl, R. Hauser, B. Wilson, M. Guinness, M. Jackson, E. De Souza; Bionel Inc, Danbury, USA.

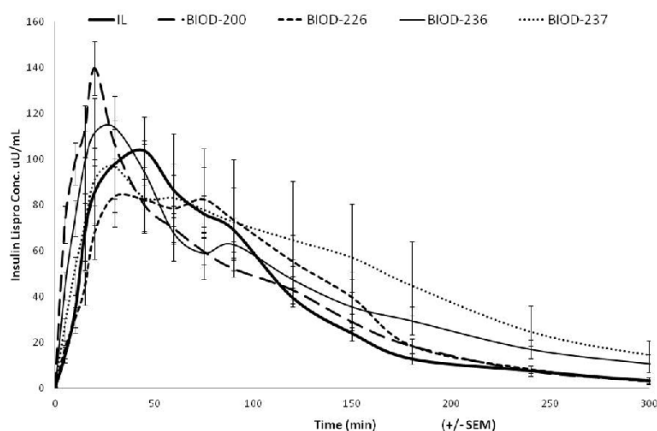
**Background and aims:** Insulin formulations containing EDTA (to sequester the zinc and destabilize the insulin hexamers into monomers/dimers) and citrate (C; to prevent re-association of the monomers by reducing the surface charge) have been effectively used to increase the rate of subcutaneous (sc) absorption of recombinant human insulin (RI) in man when compared to commercial formulations of recombinant human insulin (RHI) or insulin lispro (IL). Furthermore, adding both these excipients to rapid-acting insulin analogs such as IL, aspart or glulisine have been shown to statistically improve the rate of sc absorption relative to the corresponding insulin analogs in diabetic swine. Since the rapid-acting insulin analogs are "less stable" hexamers than RI, it was hypothesized that less EDTA and/or C alone may be sufficient to speed up the rate of absorption. The aim of this study was to use IL as the prototype insulin analog in combination with varying concentrations of EDTA and/or C to optimize the rate of sc absorption in diabetic pigs.

**Materials and methods:** Test formulations consisted of IL, BIOD-200 (IL + 100% EDTA + 100% C), BIOD-226 (IL + 0% EDTA + 100% C), BIOD-236 (IL + 25% EDTA + 87% C) and BIOD-237 (IL + 25% EDTA + 43% C). On the morning of the study, miniature diabetic swine were given a sc dose (0.25 U/kg) of test formulations followed by a meal. Blood glucose and plasma insulin were sampled from -30 to 360 min post dose. Plasma insulin was measured by an ELISA method. The time to maximal concentration ( $T_{max}$ ) and half maximal concentration ( $T_{50\%}$  early,  $T_{50\%}$ ) were calculated for each swine. Results of test formulations were compared to IL.

**Results:** IL dosed to 10 swine had a  $T_{50\%}$  of  $17.6 \pm 3.0$  and  $T_{max}$  of  $35.5 \pm 4.6$  min, which was improved to  $5.4 \pm 0.5^{**}$  and  $22.0 \pm 2.6^*$  min using 100% EDTA and 100% C (BIOD-200,  $n=10$ ;  $*p<0.05$ ,  $**p<0.005$  vs IL). BIOD-226 ( $n=9$ ), with no EDTA, had  $T_{50\%}$  and  $T_{max}$  values of  $15.5 \pm 1.6$  and  $41.1 \pm 7.1$  min, respectively which were comparable to IL demonstrating the need for EDTA. Addition of 25% EDTA + 87% C (BIOD-236;  $n=10$ ) improved the profile to  $9.0 \pm 1.7^*$  ( $T_{50\%}$ ;  $p<0.05$  vs IL) and  $27.0 \pm 4.2$  ( $T_{max}$ ) min. However, reduction of C to 43% (BIOD-237,  $n=4$ ) prolonged the times to  $11.5 \pm 2.5$  ( $T_{50\%}$ ) and  $31.3 \pm 10.1$  ( $T_{max}$ ) min. Concentration vs. time profiles of the first 300 min in diabetic miniature swine are shown in the figure below.

**Conclusion:** Both chelation of zinc ions with EDTA (to disassemble the less stable insulin analog hexamer) and C (to mask the surface charge and prevent re-aggregation) are required to enhance the sc rate of absorption of IL.

Insulin Concentration vs. Time



### 907

**Human hyaluronidase + rapid analogue insulin (RAI) improves postprandial glycaemic control in type 2 diabetes compared to insulin lispro alone**

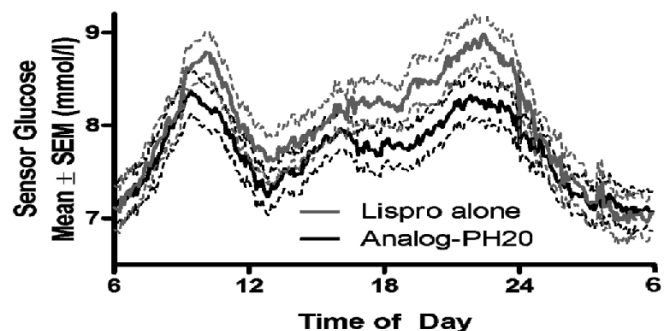
D.C. Klonoff<sup>1</sup>, R. Bergenstal<sup>2</sup>, T. Bailey<sup>3</sup>, D.E. Vaughn<sup>4</sup>, D.B. Muchmore<sup>4</sup>; <sup>1</sup>Mills-Peninsula Diabetes Research Institute, San Mateo, <sup>2</sup>International Diabetes Center, Minneapolis, <sup>3</sup>Advanced Metabolic Care and Research, Escondido, <sup>4</sup>Halozyne Therapeutics, San Diego, USA.

**Background and aims:** Recombinant human hyaluronidase (rHuPH20) accelerates the absorption and action of prandial insulins and reduces postprandial glycemic excursions following liquid test meal administration without increasing the risk of hypoglycemia. Our objective was to compare glycemic control in T2DM using prandial lispro or RAI+rHuPH20 (Analog-PH20) in the outpatient treatment setting.

**Materials and methods:** After a 4-6 week run-in using prandial glulisine + bid glargine, 121 subjects (age  $59 \pm 9.9$  years, BMI  $35.2 \pm 4.4$  kg/m<sup>2</sup>, A1C  $7.1 \pm 0.5\%$ ) were randomized (double blind 2-way crossover) to lispro+rHuPH20 or aspart+rHuPH20 vs lispro alone for two 12 week intensive management periods. Prandial doses were given immediately before meals. The primary endpoint was A1C noninferiority (0.4% margin); postprandial excursions were measured by self monitoring of blood glucose (SMBG) and by continuous glucose monitoring. Overall hypoglycemia was self-reported as SMBG  $\leq 3.9$  mmol/l or symptoms.

**Results:** Changes from baseline A1C were comparable ( $-0.48\%$  for Analog-PH20;  $-0.46\%$  for lispro), with no significant treatment difference (95% CI  $-1.2, +0.5$ ). At the end of treatment, fasting glucose values were similar between treatments (Analog-PH20  $6.85 \pm 1.47$  mmol/l vs lispro  $7.06 \pm 1.49$  mmol/l;  $p=.07$ ). Mean postmeal (90 min) excursions were reduced by 21% ( $p=.0001$ ) over the 12 week period with more subjects consistently (at least 2/3 of meals) achieving values  $<7.8$  mmol/l at breakfast (56.3% vs 35.7%,  $p=.0006$ ) and  $<7.8$  mmol/l at all meals (45.2% vs 28.0%,  $p=.0007$ ). Continuous monitoring over 3 days at the end of treatment showed improved excursion profiles with reduced glycemia throughout daytime hours (Figure). Hypoglycemia rates were comparable between treatments (7.92 episodes  $\leq 3.9$  mmol/l and 1.99 episodes  $<3.1$  mmol/l per subject-month for Analog-PH20 vs 7.66 and 1.78 episodes, respectively for lispro alone,  $p>.25$ ). Total daily insulin dose at the end of treatment ( $123 \pm 67$  for Analog-PH20 vs  $127 \pm 69$  U for lispro,  $p=.31$ ) and amount of weight gain (difference between treatments: 0.21 kg,  $p=.43$ ) were numerically lower for Analog-PH20 compared to lispro alone. Adverse event rates were comparable, and Analog-PH20 was well tolerated. Immunogenicity results showed 4 subjects with pre-existing anti-rHuPH20 antibodies; 2 subjects developed *de novo* anti-rHuPH20 antibodies without any associated adverse events.

**Conclusion:** Compared to insulin lispro alone, coformulation of RAI+rHuPH20 improves postprandial glycemic excursions while maintaining comparable A1C outcomes in an intensive diabetes treatment program.



Clinical Trial Registration Number: NCT01194258



## 908

**Human hyaluronidase provides consistent ultrafast insulin absorption and action over 3 days of continuous subcutaneous infusion**D. Muchmore<sup>1</sup>, L. Morrow<sup>2</sup>, M. Hompesch<sup>2</sup>, D.E. Vaughn<sup>1</sup><sup>1</sup>Halozyne Therapeutics, Inc., San Diego, USA, <sup>2</sup>Profil Institute for Clinical Research, Chula Vista, USA.

**Background and aims:** Rapid acting insulin analogs (RAI) demonstrate systematic variation in insulin absorption and action as infusion sites age. Euglycemic clamp studies were conducted to test the effect of human hyaluronidase (rHuPH20) on the variability of insulin aspart absorption and action as a function of infusion site age.

**Materials and methods:** Euglycemic clamps following bolus infusion of insulin aspart (100 U/ml, 0.15 U/kg) were performed as a function of infusion site age in 2 cohorts of generally healthy adult type 1 pump patients in a randomized, double blind, crossover study. In one cohort rHuPH20 (600 U/ml) was coformulated ("Coform" cohort) with insulin aspart (100 U/ml), while in the other cohort rHuPH20 (150 U in 1 ml) was injected through the new infusion set immediately after insertion and prior to any insulin infusion ("Pre-Rx" cohort).

**Results:** Consistent with previous reports, absorption kinetics and insulin action of RAI alone varied considerably with infusion site age; the fraction of insulin exposure occurring within 1 hr following bolus infusion was as little as 15% for a new infusion site and doubled to ~30% after 3 days of use. Similarly, both the onset (early  $t_{GIR50\%}$ ) and duration (calculated analogous to Mean Residence Time) of insulin action varied by up to 30 minutes as the infusion site aged. Coformulation of insulin aspart with rHuPH20 accelerated insulin absorption and action across infusion site use relative to insulin aspart alone, although the early exposure did change from ½ day to 2½ days of continuous infusion of the coformulation. Preadministration of rHuPH20 provided a consistent ultrafast profile over 3 days of infusion site use, eliminating the variability in insulin absorption and action associated with infusion site aging.

**Conclusion:** rHuPH20 accelerates insulin absorption and action following bolus infusion and reduces the variability in insulin absorption and action kinetics associated with infusion site aging.

Cohort	Infusion Site Age	Insulin Aspart Alone			With rHuPH20		
		Early Exposure (% of Total)	Onset of Action (min)	Duration of Action (min)	Early Exposure (% of Total)	Onset of Action (min)	Duration of Action (min)
Coform	1/2 day	21	47	164	35	35	147
Coform	2 1/2 days	33	35	147	51	40	133
Pre-Rx	<2 h	15	60	180	31	34	139
Pre-Rx	1 day	22	34	164	37	32	134
Pre-Rx	3 days	27	30	156	32	31	146

Clinical Trial Registration Number: NCT01275131

## 909

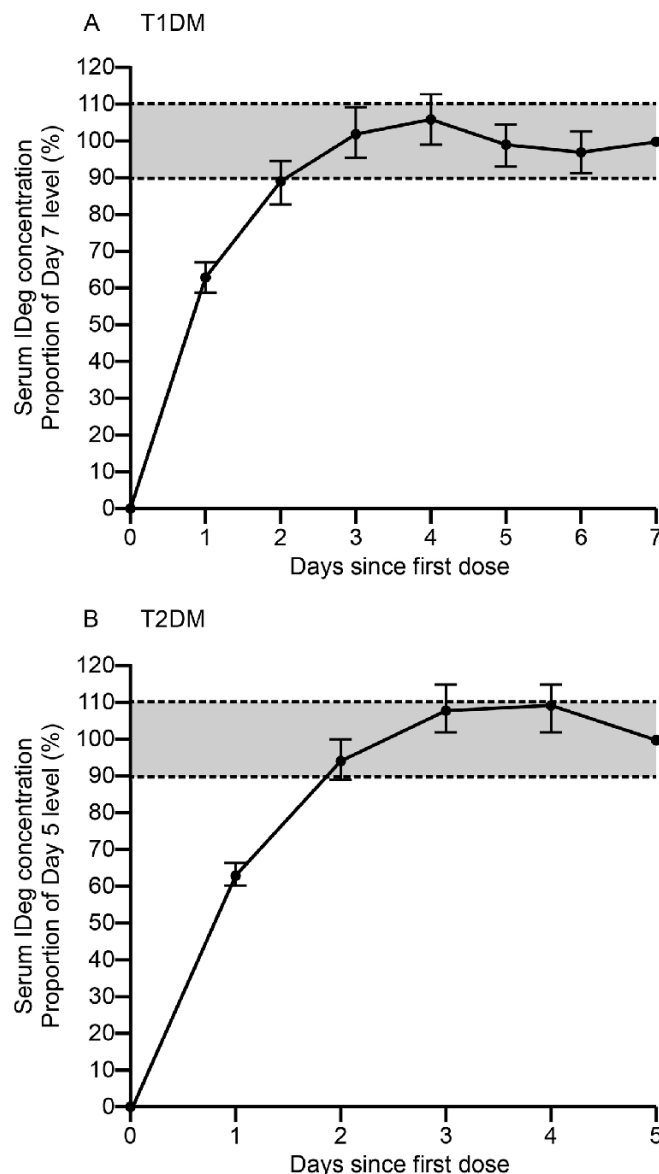
**Steady state is reached within two to three days of once-daily administration of ultra-long-acting insulin degludec**H.-V. Coester<sup>1</sup>, T. Heise<sup>1</sup>, L. Nosek<sup>1</sup>, C. Roepstorff<sup>2</sup>, S. Segel<sup>3</sup>, N. Lassota<sup>2</sup>, H.L. Haahr<sup>2</sup><sup>1</sup>Profil Institut für Stoffwechselforschung, Neuss, Germany, <sup>2</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>3</sup>Novo Nordisk A/S, Aalborg, Denmark.

**Background and aims:** The objective of basal insulin therapy is to ensure continuous insulin coverage throughout the 24 hours of the day. When insulins with a duration of action of 24 hours or less are used once daily, they are characterised by action profiles with periods of low action rising to a peak/plateau followed by a decline. Such profiles will only provide partial basal coverage implying clinical challenges in ensuring consistent glucose control throughout 24 hours. Insulin degludec (IDeg) has a duration of action extending beyond 42 hours leading to a flat and stable action profile at steady state. It is important for clinical evaluation at initiation and titration of treatment to estimate the time to reach steady state with IDeg.

**Materials and methods:** Subjects with type 1 and type 2 diabetes from two separate randomised, double-blind trials, (T1D/T2D;  $n=66/49$ ; age 37/59 years, BMI 25/30 kg/m<sup>2</sup>, HbA<sub>1c</sub> 8.1/7.6%) received IDeg at 0.4, 0.6 or 0.8 U/

kg for 8 (T1D) or 6 (T2D) days. Blood samples were taken before each dosing to determine the serum IDeg concentration on each day relative to the serum IDeg concentration before dosing on Day 7 (T1D) or 5 (T2D). The clinically relevant time to steady state was estimated as time from first dose until serum IDeg trough concentrations exceeded 90% of the final plateau level. **Results:** For all subjects, independent of dose or type of diabetes, steady state was reached after 2-3 days of IDeg dosing (Fig. 1). At steady state, exposure of IDeg was unchanged from day to day. **Conclusion:** Steady-state kinetics were observed with stable serum IDeg concentrations reached within 2-3 days of once-daily dose administration with no further increase in exposure thereafter.

**Fig 1. Relative serum IDeg trough concentrations (estimated ratios and 95% CIs) during initiation of once-daily dosing in subjects with T1DM (A) and T2DM (B).**



Clinical Trial Registration Number: NCT01154881 and NCT01114542  
Supported by: Novo Nordisk

## 910

**Altering the timing of once-daily dosing of insulin degludec achieves similar glycaemic control and safety to dosing at the same time each day in patients with type 1 diabetes**

D. Russell-Jones<sup>1</sup>, P. Hollander<sup>2</sup>, B. Miranda-Palma<sup>3</sup>, J.G. Cooper<sup>4</sup>, E. Franek<sup>5</sup>, S. Bain<sup>6</sup>, C.B. Djurhuus<sup>7</sup>, S.C. Tamer<sup>7</sup>, C. Mathieu<sup>8</sup>;

<sup>1</sup>Royal Surrey County Hospital & University of Surrey, Guildford, UK, <sup>2</sup>Baylor University Medical Center, Dallas, USA, <sup>3</sup>University of Miami, Miller School of Medicine, USA, <sup>4</sup>Stavanger University Hospital, Norway, <sup>5</sup>Central Clinical Hospital MSWiA, Warsaw, Poland, <sup>6</sup>Abertawe Bro Morgannwg University NHS Trust, Singleton Hospital, Swansea, UK, <sup>7</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>8</sup>UZ Gasthuisberg, KU Leuven, Belgium.

**Background and aims:** To date, consistent timing of basal insulin injection is needed to ensure stable day-to-day glycaemic control. Insulin degludec (IDeg) provides an ultra-long, flat action profile, enabling flexible dosing to accommodate an individual patient's lifestyle. The primary objective of this study was to confirm the efficacy of IDeg administered in a flexible dose regimen (IDeg Flex). A secondary objective and the topic of this report was to compare whether a flexible dosing regimen (IDeg Flex) provided comparable safety and efficacy to IDeg dosed at the same time each day (IDeg OD).

**Materials and methods:** In this 26-week, open-label, randomised, treat-to-target trial in patients with type 1 diabetes, IDeg OD (n=165) administered with the evening meal, was compared to a forced-flexible dosing regimen of IDeg Flex (n=164) where subjects alternated insulin administration timing between morning and evening to create intervals of a minimum of 8 and a maximum of 40 hours between insulin doses.

**Results:** Mean baseline values for HbA<sub>1c</sub> (both groups 7.7%), fasting plasma glucose (FPG) (9.6 vs. 10.0 mmol/L), disease duration (17.3 vs. 20.0 years), age (42.6 vs. 44.5 years), and BMI (27.0 vs. 26.4 kg/m<sup>2</sup>) were comparable between IDeg Flex and IDeg OD groups, respectively. At 26 weeks, 84% of individuals in both groups completed the trial. Both IDeg Flex and IDeg OD reduced HbA<sub>1c</sub> by 0.4%-points (estimated treatment difference [ETD] IDeg Flex-IDeg OD: 0.01% [95% CI: -0.13; 0.14]). Observed FPG reductions were greater with IDeg OD (-1.28 mmol/L with IDeg Flex and -2.54 mmol/L with IDeg OD (ETD IDeg Flex-IDeg OD: 0.95 mmol/L [95% CI: 0.15; 1.75])). Overall hypoglycaemia rates (PG <3.1 mmol/L or severe hypoglycaemia requiring assistance) were similar between IDeg Flex and IDeg OD groups (82.4 vs. 88.3 events/patient-year, respectively; estimated rate ratio (ERR) IDeg Flex:IDeg OD 0.92 [95% CI: 0.76; 1.12])). Rates of nocturnal hypoglycaemia (time of onset between 00:01 and 05:59 hours, inclusive) were significantly lower with IDeg Flex compared with IDeg OD (6.2 vs. 9.6 events/patient-year, respectively; ERR: 0.63 [95% CI: 0.46; 0.86])). Severe hypoglycaemia rates were similar for both groups, as were adverse event rates. Final IDeg doses were 0.42 (IDeg Flex) and 0.38 (IDeg OD) U/kg per day.

**Conclusion:** IDeg can be administered in a once-daily regimen but at a different time from day to day with no difference in glycaemic control or safety compared to standard OD dosing in patients with type 1 diabetes. Greater dosing flexibility may represent a major improvement in patient convenience by allowing injection times to be changed daily according to needs of the individual.

Clinical Trial Registration Number: NCT01079234

Supported by: Novo Nordisk

## 911

**Insulin degludec allows for flexible daily dosing in type 1 diabetes, providing equal glycaemic control with less nocturnal hypoglycaemia than insulin glargine over 52 weeks**

J.G. Cooper<sup>1</sup>, C. Mathieu<sup>2</sup>, P. Hollander<sup>3</sup>, B. Miranda-Palma<sup>4</sup>, E. Franek<sup>5</sup>, S. Bain<sup>6</sup>, J. Larsen<sup>7</sup>, S.C. Tamer<sup>7</sup>, D.L. Russell-Jones<sup>8</sup>;

<sup>1</sup>Department of Medicine, Stavanger University Hospital, Stavanger, Norway, <sup>2</sup>UZ Gasthuisberg, KU Leuven, Belgium, <sup>3</sup>Baylor University Medical Center, Dallas, USA, <sup>4</sup>University of Miami, Miller School of Medicine, Miami, USA, <sup>5</sup>Central Clinical Hospital MSWiA, Warsaw, Poland, <sup>6</sup>Abertawe Bro Morgannwg University NHS Trust, Singleton Hospital, Swansea, UK, <sup>7</sup>Novo Nordisk, Søborg, Denmark, <sup>8</sup>Royal Surrey County Hospital & University of Surrey, Guildford, UK.

**Background and aims:** Current basal insulin preparations must be injected at the same time every day to ensure stable glycaemic control, particularly in patients with type 1 diabetes. Insulin degludec (IDeg) is an ultra-long-acting basal insulin that forms soluble multi-hexamers upon s.c. injection, resulting

in a flat and stable glucose-lowering effect. This profile may enable flexible intervals between daily doses. The objective of this study was to investigate whether flexible dosing of IDeg provided comparable efficacy and safety to insulin glargine (IGlar) dosed at the same time each day.

**Materials and methods:** This 26 + 26-week (main + extension period), open-label, treat-to-target trial in patients with type 1 diabetes (n=493) compared once-daily (OD) IDeg (with evening meal) or IGlar (at same time each day) to a flexible IDeg regimen (IDeg Flex), each in combination with meal-time insulin aspart. In the first 26 weeks, IDeg Flex patients were required to alternate OD insulin administration between morning and evening, thus creating intervals of a minimum of 8 and a maximum of 40 hours between doses. For the 26-week extension, IDeg OD and IDeg Flex patients were allocated to IDeg Free Flex (IDeg FF; n=329), which allowed dosing at any time of day with dose intervals between 8 and 40 hours, and compared to IGlar (n=164), which was continued as per label. Herein, we report data for IDeg FF and IGlar at 52 weeks.

**Results:** Mean baseline characteristics were comparable between groups (mean age 43.7 years, diabetes duration 18.5 years, HbA<sub>1c</sub> 7.7% and fasting plasma glucose [FPG] 9.8 mmol/L). At 52 weeks, IDeg FF and IGlar reduced baseline HbA<sub>1c</sub> by 0.13 and 0.21%-points (estimated treatment difference [ETD] IDeg FF-IGlar: 0.07% [95% CI: -0.05; 0.19])). FPG reductions were significantly greater with IDeg FF versus IGlar (ETD IDeg FF-IGlar: -1.07mmol/L [95% CI: -1.82; -0.32])). Overall hypoglycaemia rates (PG <3.1mmol/L or severe hypoglycaemia requiring assistance) were similar (68.1 vs. 63.4 events/patient-year for IDeg FF and IGlar, respectively; estimated rate ratio (ERR) IDeg FF:IGlar 1.09 [95% CI: 0.91; 1.29])). Nocturnal hypoglycaemia was significantly 25% lower for IDeg FF versus IGlar (ERR: 0.75 [95% CI: 0.58; 0.97])), and severe hypoglycaemia was 26% lower (ERR: 0.74 [95% CI: 0.38; 1.42])). Adverse event rates were low in both groups. Basal insulin doses did not differ; 0.40 (IDeg FF) and 0.42 (IGlar) U/kg at 52 weeks.

**Conclusion:** This study demonstrates that IDeg can be administered conveniently at any time of day with similar glycaemic control and less nocturnal hypoglycaemia than standard IGlar given OD at the same time each day over 52 weeks in patients with type 1 diabetes. A flexible dosing regimen could make it easier for patients to incorporate their insulin into changing daily routines and may improve patient adherence.

Clinical Trial Registration Number: NCT01079234

Supported by: Novo Nordisk

## 912

**Insulin degludec 200 U/ml is ultra-long-acting and has a flat and stable glucose-lowering effect**

U. Hövelmann<sup>1</sup>, T. Heise<sup>1</sup>, L. Nosek<sup>1</sup>, S.G. Böttcher<sup>2</sup>, H. Hastrup-Nielsen<sup>2</sup>, H.L. Haahr<sup>2</sup>;

<sup>1</sup>Profil Institut für Stoffwechselforschung, Neuss, Germany, <sup>2</sup>Novo Nordisk A/S, Søborg, Denmark.

**Background and aims:** Insulin degludec (IDeg) is a new-generation, ultra-long-acting basal insulin, which is developed as both 100 U/ml and 200 U/ml formulations in order to accommodate the wide range of insulin dose requirements characteristic of diabetes mellitus, especially type 2 diabetes. With IDeg 200 U/ml, up to 160 U of IDeg can be administered in a single injection using a newly developed prefilled pen.

**Materials and methods:** We investigated the pharmacodynamic and pharmacokinetic properties of 0.6 U/kg IDeg 200 U/ml in subjects with type 2 diabetes (n=16, mean: BMI, 30 kg/m<sup>2</sup>; HbA<sub>1c</sub>, 7.3%) who received IDeg 200 U/ml once daily over 6 days. On Day 6 while at steady state, a 26-hour euglycaemic glucose clamp was conducted (Biostator; clamp blood glucose level: 5 mmol/l).

**Results:** As previously shown for IDeg 100 U/ml, the mean glucose infusion rate (GIR) profile for IDeg 200 U/ml was flat and stable over the dosing interval tau (Fig. 1). The glucose-lowering effect of IDeg was evenly distributed over the dosing interval, with AUC<sub>GIR</sub> for each of the two 12-hour intervals being approximately 50% of the total AUC (AUC<sub>GIR,tau,SS</sub>). The effect of IDeg extended beyond 26 hours in all subjects, as blood glucose stayed close to the target level throughout the clamp. The terminal half-life at steady state was 26.2 hours. IDeg 200 U/ml was well tolerated, no injection site reactions were reported and no safety concerns were identified.

**Conclusion:** IDeg 200 U/ml has a flat and stable glucose-lowering effect in people with type 2 diabetes.

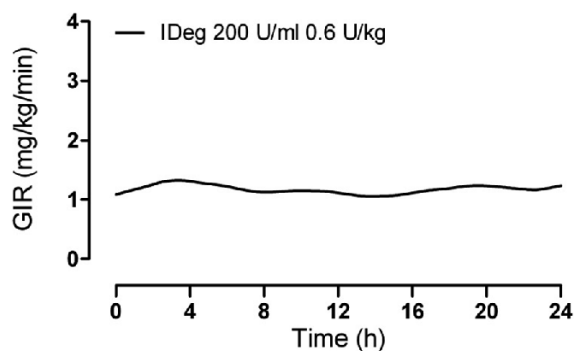


Fig. 1: Mean 24-hour GIR profile of IDeg 200 U/ml at steady state

Clinical Trial Registration Number: NCT01154881

Supported by: Novo Nordisk

## 913

**Steady-state pharmacokinetics (PK) and glucodynamics (GD) of the novel, long-acting basal insulin LY2605541 in patients with type 2 diabetes mellitus**

T. Heise<sup>1</sup>, D.C. Howey<sup>2</sup>, V.P. Sinha<sup>2</sup>, S.L. Choi<sup>2</sup>, K.F. Mace<sup>2</sup>;

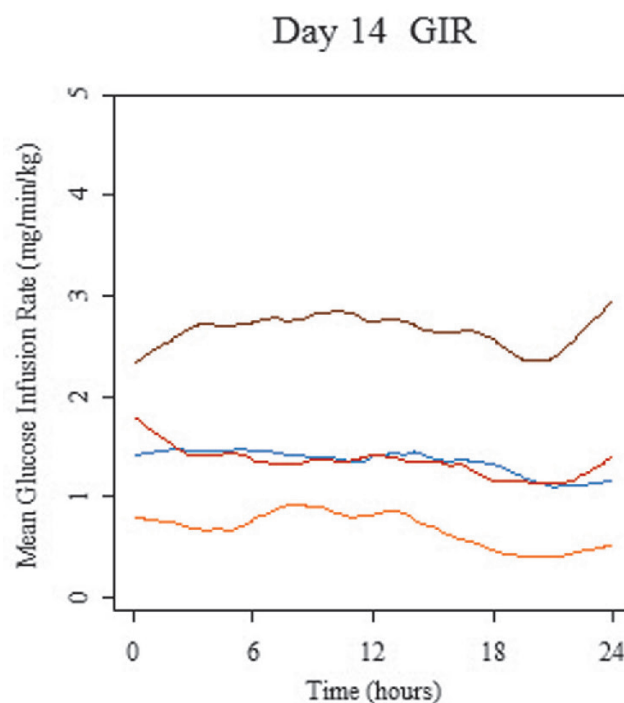
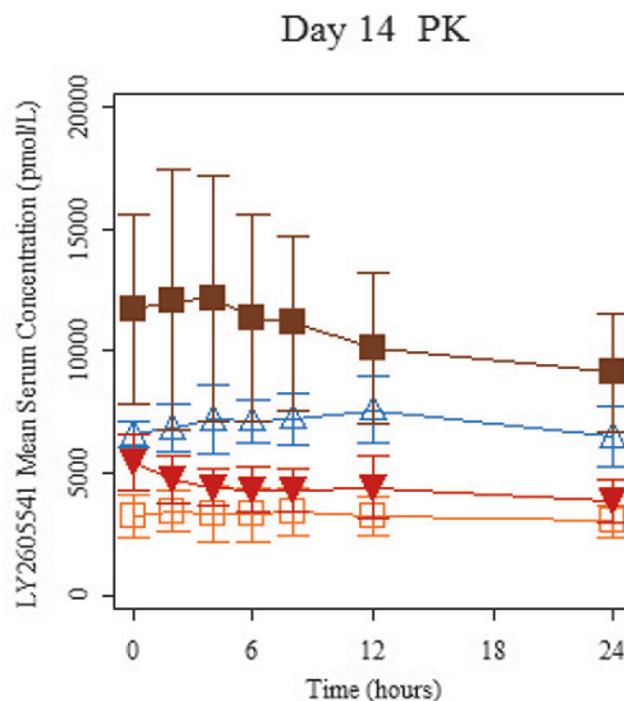
<sup>1</sup>Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany, <sup>2</sup>Eli Lilly and Company, Indianapolis, USA.

**Background and aims:** The basal insulin LY2605541 (LY) is PEGylated insulin lispro designed to have a large hydrodynamic size which delays insulin absorption and reduces clearance, resulting in prolonged duration of action. This parallel group, open-label, dose escalation study examined the PK and GD of LY after multiple-dose administration.

**Materials and methods:** Fixed-doses of LY (3-9 nmol/kg) were given once-daily (QD) for 14 days to insulin-treated patients with T2DM (N=32 [30 M/2 F; 8 patients per arm]; mean [SD] age, 56 [6] yrs; BMI, 31.0 [2.4] kg/m<sup>2</sup>; HbA1c, 7.8 [0.4] %). A 24-h euglycemic glucose clamp was conducted on Days 1 and 14.

**Results:** Pharmacokinetic steady state was achieved within 7-10 days and the peak to trough fluctuation was <1.5 which translated to a nearly "peakless" glucose infusion rate at steady state and a duration of action of at least 24 h [Figure]. Based on AUC, there was a gradual build up of mean LY concentrations (8.4-fold higher vs. single dose). Across dose levels  $t_{1/2}$  ranged from 44.7-75.5 h (~2-3 days). As steady state was achieved there were dose-dependent reductions in the prandial insulin dose and in fasting blood glucose, which decreased to 60-100 mg/dL across dose levels. The nocturnal glucose control between 3 am and 9 am was unchanged. No severe or prolonged hypoglycemia was reported. Mild hypoglycemia was the most common adverse event.

**Conclusion:** In this Phase 1 study of fixed LY doses without titration, LY was well-tolerated and demonstrated a flat PK and GD profile accompanied by glucose normalization, prandial insulin dose reduction, and no severe hypoglycemia.



— 3 nmol/kg LY2605541  
 — 6 nmol/kg LY2605541  
 — 9 nmol/kg LY2605541  
 — 4.5 nmol/kg LY2605541



## 914

**Effects of a novel PEGylated basal insulin, LY2605541, on hepatic glucose output and muscle glucose uptake: simulations based on data from euglycaemic clamp studies**

B.G. Topp<sup>1</sup>, J.S. Geiser<sup>1</sup>, D.K.W. Soon<sup>1</sup>, T. Heise<sup>2</sup>, M.D. Michael<sup>1</sup>, S.J. Jacober<sup>1</sup>, J.M. Beals<sup>1</sup>, V.P. Sinha<sup>2</sup>;

<sup>1</sup>Eli Lilly and Company, Indianapolis, USA, <sup>2</sup>Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany.

**Background and aims:** LY2605541 (LY) is insulin lispro with a 20 kDa polyethylene glycol (PEG) moiety covalently attached to lysine B28. LY is designed to have a large hydrodynamic size that slows absorption and reduces clearance, resulting in a longer duration of action. The glucodynamics have been previously assessed in two studies, a single dose study in healthy volunteers (HV) and a multiple-dose study in patients with type 2 diabetes mellitus (T2DM). This *in silico* study sought to characterize the physiologic effects and tissue distribution of LY2605541 using pharmacokinetic and pharmacodynamic data obtained from human studies in an established model of human physiology (Metabolism Physioblab® platform).

**Materials and methods:** The pharmacokinetic impact of LY's size was described using a pharmacokinetic tissue distribution model; a model was developed for both LY and insulin glargine based on transit rate through the capillaries and the lymphatic system. The effects of LY and glargine on glycemic control, hepatic glucose output (HGO) and muscle glucose uptake (MGU) were predicted using the Metabolism Physioblab® platform, a proprietary model calibrated to over 3000 papers and validated against 300 publications.

**Results:** After single doses (0.5–0.8 U/kg of insulin glargine, or 0.3–2.2 U/kg of LY) in HV, glargine concentrations were predicted to be similar in plasma, liver and muscle while LY concentrations were predicted to be higher in liver than muscle (11.2 vs. 2.4 µU/mL at 2.2 U/kg LY) due to a slower transit across the capillary bed relative to lymph flow. Glucose infusion rates (GIR) were similar (140 vs. 149 mg/min) for 0.5 U/kg insulin glargine and 2.2 U/kg LY. However, their predicted effects at the liver (ΔHGO, -52, -129 mg/min) and muscle (ΔMGU, 87, 19 mg/min), differed. After multiple, daily doses (0.33–1 U/kg) in patients with T2DM, LY gradually increased to reach steady-state concentrations (88 vs 22 µU/mL for liver vs muscle for a 0.5 U/kg dose) in approximately a week. LY predominantly inhibited HGO (GIR = 147, ΔHGO = -124, ΔMGU 24 mg/min for 0.5 U/kg). Thus, LY is predicted to regulate fasting glucose levels largely by inhibiting HGO while glargine is predicted to regulate fasting glycemia largely by inhibition of HGO and stimulation of MGU.

**Conclusion:** These simulation results suggest that LY2605541 has a preferential hepatic effect and its effect on MGU is predicted to be less than insulin glargine. Clinical trials are planned to test these model predictions.

## 915

**Liver preferential effects of an insulin analogue demonstrated by insulin receptor phosphorylation and glucose dynamics in insulin target tissues *in vivo***

C.L. Brand, S.D. Bouman, J. Kildegaard, T.B. Kjeldsen, P. Madsen, H. Naver, C.B. Jeppesen, J.J. Fels, J. Sturis, E. Nishimura;  
Diabetes Research Unit, Novo Nordisk A/S, Maaloev, Denmark.

**Background and aims:** Compared to the distribution of endogenously secreted insulin throughout the body, subcutaneous (sc) insulin is distributed in a non-physiological manner to the insulin target tissues with the peripheral tissues being relatively overexposed to insulin compared to the liver, resulting in increased risk of hypoglycaemia. The present study investigates a novel insulin analogue (insulin 327) and its potential liver preferential effects compared to human insulin (HI) in rodents.

**Materials and methods:** Insulin receptor phosphorylation (pIR) was measured in liver, muscle and fat tissues at 0, 5, 15, 60, 120 and 180 min after intravenous (iv) injection of either HI or insulin 327 in conscious male NMRI mice. The dynamic rates of glucose uptake (GU) and hepatic glucose production (HGP) were determined by <sup>3</sup>H-glucose techniques during 5 hrs of three different constant iv infusion rates of either HI or insulin 327 and variable glucose infusion rates (GIR) to maintain euglycemic clamp in conscious male Sprague Dawley rats. The potency of insulin 327 relative to HI was calculated as the ratio between their ED<sub>50</sub> values calculated by fitting the insulin infusion rates and the corresponding response values to Sigmoidal curves.

**Results:** For similar glucose lowering in mice, insulin 327 exerted a liver preferential pIR pattern as the response in liver accounted for 71% of the com-

bined pIR response (AUC) in all tissues compared to 43% for HI. The potency of insulin 327 on GIR during the last hr of the clamp in rats was 59% relative to HI. The potency of insulin 327 relative to HI on ΔAUCs for HGP and GU was 77% and 37%, respectively, indicating a more potent suppression of HGP than stimulation of GU.

**Conclusion:** These data demonstrate that insulin 327 exerts liver preferential pIR and glucose dynamic patterns compared to HI in rodents, and may therefore represent a novel basal insulin regimen with a potential for improving the hypoglycaemia safety margin.

## PS 074 New insulins II

### 916

#### Better glycaemic control and weight loss with the novel long-acting PEGylated basal insulin LY2605541 compared with insulin glargine in patients with type 1 diabetes

T. Blevins<sup>1</sup>, J. Rosenstock<sup>2</sup>, R.M. Bergenstal<sup>3</sup>, L.A. Morrow<sup>4</sup>, M.J. Prince<sup>5</sup>, Y. Qu<sup>5</sup>, V.P. Sinha<sup>5</sup>, D.C. Howey<sup>6</sup>, S.J. Jaber<sup>6</sup>;  
<sup>1</sup>Texas Diabetes and Endocrinology, Austin, <sup>2</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, <sup>3</sup>International Diabetes Center at Park Nicollet, Minneapolis, <sup>4</sup>Profil Institute for Clinical Research, Inc, Chula Vista, <sup>5</sup>Eli Lilly and Company, Indianapolis, <sup>6</sup>Eli Lilly and Company, retired, Indianapolis, USA.

**Background and aims:** The basal insulin analogue LY2605541 (LY) is PEGylated insulin lispro designed to have a large hydrodynamic size which delays insulin absorption and reduces clearance, resulting in prolonged duration of action. Primary aim of this Phase 2, randomised, open-label, 2x2 crossover study was to determine if LY was noninferior (margin of 0.6 mmol/L) to glargine (GL) for daily mean blood glucose (BG) in treatment of T1D.

**Materials and methods:** Patients (N=137) received basal insulin (LY or GL) once daily, plus prandial insulin, for 8 weeks, followed by crossover treatment for 8 weeks. Daily mean BG was obtained from three 8-point self-monitored BG profiles (pre- and 2 hrs post-meal, at bedtime, and 3 AM) the week prior to each visit.

**Results:** After 8 weeks, LY was non-inferior and also superior to GL in daily mean BG ( $\pm$  SEM:  $8.01 \pm 0.14$  vs  $8.43 \pm 0.17$  mmol/L, LS mean difference =  $-0.55$  mmol/L; 90% CI  $[-0.81, -0.29]$ ,  $p < .001$ ). Fasting BG variability (SD;  $2.70 \pm 0.10$  vs  $3.16 \pm 0.12$  mg/dL,  $p < .001$ ) and  $HbA_{1c}$  ( $-0.59$  vs  $-0.43\%$ ,  $p < .001$ ) were reduced with LY compared with GL. Prandial insulin dose was reduced during LY treatment (from 0.225 to 0.187 U/kg/day) and increased (to 0.242 U/kg/day) with GL ( $p < .001$ ). LY was associated with weight loss and GL with weight gain (LS means  $-1.2$  vs  $+0.7$  kg,  $p < .001$ ). Total hypoglycaemia rate was higher for LY (8.7 vs 7.4 events/30 days,  $p = .04$ ), but nocturnal hypoglycaemia rate was lower (0.9 vs 1.1 events/30 days,  $p = .01$ ). Adverse events (including severe hypoglycaemia) were similar, although more mild gastrointestinal-related events (dyspepsia, nausea, abdominal distension) occurred with LY (15 vs 4%,  $p < .001$ ). During LY treatment, mean changes were higher for ALT, AST, triglycerides and LDL-cholesterol and lower for HDL-cholesterol compared to GL (all  $p < .02$ ), but all mean changes remained within normal range.

**Conclusion:** LY provided greater improvements in glycaemic control and reduced nocturnal hypoglycaemia vs GL in T1D. Notably, this was accompanied by reduced weight and lowering of prandial insulin doses.

*Clinical Trial Registration Number:* NCT01049412

*Supported by:* Eli Lilly and Company

### 917

#### Weight loss and lower nocturnal hypoglycaemia with novel long-acting basal insulin LY2605541 versus insulin glargine in patients with type 2 diabetes

R.M. Bergenstal<sup>1</sup>, J. Rosenstock<sup>2</sup>, R.F. Arakaki<sup>3</sup>, M.J. Prince<sup>4</sup>, Y. Qu<sup>4</sup>, V.P. Sinha<sup>4</sup>, D.C. Howey<sup>4</sup>, S.J. Jaber<sup>4</sup>;  
<sup>1</sup>International Diabetes Center at Park Nicollet, Minneapolis, <sup>2</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, <sup>3</sup>University of Hawaii at Manoa, Honolulu, <sup>4</sup>Eli Lilly and Company, Indianapolis, USA.

**Background and aims:** The basal insulin analogue LY2605541 (LY) is PEGylated insulin lispro designed to have a large hydrodynamic size which delays insulin absorption and reduces clearance, resulting in prolonged duration of action. This 12-week, Phase 2, randomised, open-label, parallel study evaluated whether self-monitored fasting blood glucose (FBG) was lower with once-daily LY than insulin glargine (GL).

**Materials and methods:** Patients converted to AM insulin administration during lead-in and were randomised 2:1 to AM administration of LY (n=195) or GL (n=93) from basal insulin (GL: n=248, NPH insulin: n=39).

**Results:** At baseline, for LY vs. GL, mean  $\pm$  SEM FBG was  $8.149 \pm 0.16$  vs.  $7.79 \pm 0.23$  mmol/L,  $HbA_{1c}$  was  $7.7 \pm 0.1$  vs.  $7.8 \pm 0.1\%$ , and weight was  $90.7 \pm 1.4$  vs.  $89.7 \pm 2.1$  kg. At Week 12, LY vs. GL resulted in similar mean FBG ( $6.56 \pm 0.11$  vs.  $6.49 \pm 0.15$  mmol/L,  $p = .433$ ), and mean  $HbA_{1c}$  ( $7.0 \pm 0.06$  vs.  $7.2 \pm 0.09\%$ ,  $p = .279$ ). Intra-day blood glucose variability, as measured by 8-point self-monitored blood glucose standard deviation, was reduced with LY (1.91

$\pm 0.06$  vs.  $2.17 \pm 0.10$  mmol/L,  $p = .031$ ). LY patients had significant mean weight loss ( $0.58 \pm 0.16$  kg,  $p = .007$ ), while GL patients gained weight ( $0.31 \pm 0.18$  kg,  $p = .66$ ; least-squares mean difference:  $-0.84$  kg,  $p = .001$ ). Mean hypoglycaemia rate (#/30d) with LY was not different compared to GL (total: 1.34 vs. 1.52;  $p = .80$ ; nocturnal: 0.25 vs. 0.39,  $p = .18$ ). After adjusting for baseline nocturnal hypoglycaemia, LY patients had a 48% rate reduction in nocturnal hypoglycaemia events compared to GL ( $p = .021$ ). Adverse events were similar across treatments. Mean increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were higher with LY versus GL ( $p < .01$ ), but remained in normal range. Triglyceride (TG) change from baseline was not significant for either group, but endpoint TGs were higher with LY vs. GL ( $p < .01$ ). There was no difference in LDL-C or HDL-C between treatments.

**Conclusion:** In conclusion, LY provided similar glycaemic control, with reduced intra-day variability and lower nocturnal hypoglycaemic events compared to GL in patients with type 2 diabetes. While improving glycaemic control, LY resulted in weight loss.

*Clinical Trial Registration Number:* NCT01027871

*Supported by:* Eli Lilly and Company

### 918

#### Contrasting weight changes with LY2605541, a novel long-acting insulin, and insulin glargine despite similar improved glycaemic control in type 1 diabetes and type 2 diabetes

S.J. Jaber<sup>1</sup>, J. Rosenstock<sup>2</sup>, R.M. Bergenstal<sup>3</sup>, M.J. Prince<sup>1</sup>, Y. Qu<sup>1</sup>, J.M. Beals<sup>1</sup>;

<sup>1</sup>Eli Lilly and Company, Indianapolis, <sup>2</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, <sup>3</sup>International Diabetes Center at Park Nicollet, Minneapolis, USA.

**Background and aims:** The basal insulin analogue LY2605541 (LY) is a PEGylated insulin lispro designed to have a large hydrodynamic size which delays insulin absorption and reduces clearance, resulting in prolonged duration of action. Improved glycaemic control with insulin generally results in weight gain. However, once-daily LY showed glycaemic control comparable with or superior to insulin glargine (GL), and resulted in weight loss compared with weight gain with GL.

**Materials and methods:** Weight change was examined in 2 Phase 2, randomised, open-label trials comparing LY and GL: a 12-wk study in patients (N=288) with type 2 diabetes (T2D) and an 8-wk 2x2 crossover study in patients (N=137) with type 1 diabetes (T1D).

**Results:** In the T2D trial, patients lost 0.6 kg with LY and gained 0.3 kg with GL (resulting in a treatment difference of  $-0.84$  kg,  $p < .01$ ). Weight loss was more common with LY than GL (57% vs 40%,  $p = .01$ ) and loss of  $\geq 5\%$  body weight was more frequent with LY than GL (5% vs 0%; respectively,  $p = .03$ ) with no correlation between baseline BMI and mean weight change. Higher insulin doses correlated with less weight loss with LY ( $p < .01$ ) and greater weight gain with GL ( $p < .01$ ). There was no correlation between hypoglycaemia rate and weight change with LY ( $p = .28$ ). In the T1D trial, patients lost 1.2 kg with LY and gained 0.7 kg with GL (resulting in a treatment difference of  $-1.9$  kg,  $p < .01$ ). Weight loss was more common during LY treatment (66% vs 40%,  $p < .01$ ) as was loss of  $\geq 5\%$  body weight (12% vs 1%;  $p < .01$ ) with no correlation between weight and baseline BMI or dose for either insulin. Prandial insulin dose decreased (0.23 U/kg/day to 0.19 U/kg/day) with LY and increased to 0.24 U/kg/day with GL (between-treatment  $p < .01$ ). More mild gastrointestinal (GI) adverse events were reported with LY, but LY-treated patients with GI events had less weight loss (0.84 kg) than those without (1.33 kg). There was no correlation between hypoglycaemia rate and weight change with LY ( $p = .65$ ).

**Conclusion:** Improved glycaemic control with long-acting basal insulin analogue LY is associated with weight loss that is not dependent on baseline BMI or rate of hypoglycaemic events.

*Clinical Trial Registration Number:* NCT01027871 and NCT01049412

*Supported by:* Eli Lilly and Company

## 919

**Lower glucose variability and hypoglycaemia measured by continuous glucose monitoring with novel long-acting insulin LY2605541 versus insulin glargine**4E.J. Bastyr<sup>1</sup>, R.M. Bergenstal<sup>2</sup>, J. Rosenstock<sup>3</sup>, M.J. Prince<sup>1</sup>, Y. Qu<sup>1</sup>, S.J. Jacober<sup>1</sup>;<sup>1</sup>Eli Lilly and Company, Indianapolis, <sup>2</sup>International Diabetes Center at Park Nicollet, Minneapolis, <sup>3</sup>Dallas Diabetes and Endocrine Center at Medical City, Dallas, USA.

**Background and aims:** The basal insulin analogue LY2605541 (LY) is PE-Glylated insulin lispro designed to have a large hydrodynamic size which delays insulin absorption and reduces clearance, resulting in prolonged duration of action. Hypoglycaemia and glucose variability were assessed with continuous glucose monitoring (CGM) of interstitial glucose (IG) in a subset of patients with type 2 diabetes from a Phase 2, randomised, open-label, parallel study of LY (n=51) or insulin glargine (GL, n=25).

**Materials and methods:** CGM was conducted on 3 consecutive days (72–84 hrs) during the week before Week 0, 6, and 12 study visits, and IG intra-patient, intra- and inter-day standard deviations (SD) during nocturnal (2400–0600 hrs) and diurnal (0600–2400 hrs) periods were calculated to assess glucose variability. A hypoglycaemia episode was defined as IG  $\leq$  3.9 mmol/L and continued until IG  $>$  3.9 mmol/L for 15 min (or 3 time points), and all statistical comparisons were tested at 2-sided alpha = 0.1.

**Results:** At 12 weeks, LY-treated patients spent less time with IG below 3.9 mmol/L than GL-treated patients during the nocturnal period ( $11 \pm 5$  vs.  $38 \pm 13$  min,  $p=.024$ ) and during the 24-hr period ( $25 \pm 6$  vs.  $83 \pm 16$  min,  $p<.001$ ). Significantly fewer LY- than GL-treated patients experienced any hypoglycaemia (50.0% vs. 78.3%,  $p=.036$ ), including nocturnal hypoglycaemia (20.5% vs. 47.8%,  $p=.027$ ), based on CGM. At 12 weeks, both treatments resulted in similar mean glucose values, as indicated by the area under the IG curve, during the 24-hr period (LY: 11601 mmol/L•min; GL: 11286 mmol/L•min). LY-treated patients had significantly lower intra-day glucose SD at 12 weeks compared to GL-treated patients for both nocturnal ( $1.00 \pm 0.07$  vs.  $1.35 \pm 0.16$  mmol/L,  $p=.061$ ) and diurnal ( $2.03 \pm 0.10$  vs.  $2.50 \pm 0.18$  mmol/L,  $p=.039$ ) periods.

**Conclusion:** In conclusion, by CGM, LY treatment compared to GL resulted in: less time spent in hypoglycaemia, fewer patients experiencing hypoglycaemia, and lower intra-day glucose variability.

*Clinical Trial Registration Number:* NCT01027871

*Supported by:* Eli Lilly and Company

## 920

**Reduced nocturnal hypoglycaemia with insulin degludec as compared to insulin glargine: results of a 2-year randomised trial in type 2 diabetes**H.W. Rodbard<sup>1</sup>, B. Zinman<sup>2</sup>, B. Cariou<sup>3</sup>, A. Philis-Tsimikas<sup>4</sup>, Y. Handelsman<sup>5</sup>, T.V. Skjoth<sup>6</sup>, P.-L. Chu<sup>7</sup>, C. Mathieu<sup>8</sup>;<sup>1</sup>Endocrine and Metabolic Consultants, Rockville, USA, <sup>2</sup>Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Canada,<sup>3</sup>Department of Endocrinology, Nantes University Hospital, Nantes, France,<sup>4</sup>Scripps Whittier Diabetes Institute, La Jolla, USA, <sup>5</sup>Metabolic Institute of America, Tarzana, <sup>6</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>7</sup>Novo Nordisk Inc., Princeton, USA, <sup>8</sup>UZ Gasthuisberg, KU Leuven, Belgium.

**Background and aims:** This 2-year (yr), open-label, treat-to-target, non-inferiority study compared the efficacy and safety of the new ultra-long-acting basal insulin degludec (IDeg) to glargine (IGlar), when used in combination with OADs in type 2 diabetes mellitus (T2DM).

**Materials and methods:** The study consisted of a 1-yr initial study period followed by a 1-yr extension, with subjects maintaining the randomisation treatment assignment throughout. Subjects were randomised 3:1 to IDeg or IGlar given s/c once daily in combination with metformin  $\pm$  DPP-4 inhibitor. Basal insulin was titrated to achieve pre-breakfast, plasma-corrected, self-monitored blood glucose targets of 3.9–4.9 mmol/L. Statistical analyses of glycaemic efficacy endpoints, hypoglycaemia, and body weight were performed following the intention-to-treat principle. HbA<sub>1c</sub> results from the extension trial set (ETS), including subjects receiving at least one dose of IDeg or IGlar in the extension period, are also presented. Data at the end of 2 yrs of treatment [EOT] are presented.

**Results:** Of 1030 subjects (mean age 59 yrs; diabetes duration 9.2 yrs; HbA<sub>1c</sub> 8.2%; fasting plasma glucose [FPG] 9.7 mmol/L), 725 entered the extension and 659 (IDeg: 505; IGlar: 154) completed 2 yrs of treatment. Mean observed

HbA<sub>1c</sub> reduction at EOT was similar between the two groups: 1.0% (IDeg) vs 1.1% (IGlar); estimated treatment difference [ETD] IDeg-IGlar: 0.12% [95%CI -0.01; 0.25],  $p=NS$ ; ETS: 1.1% vs 1.2%; ETD: 0.07% [-0.07; 0.22],  $p=NS$ . A similar proportion of subjects achieved HbA<sub>1c</sub>  $<$ 7% (IDeg: 47%; IGlar: 53%;  $p=NS$ ). Mean observed FPG reduction was greater with IDeg: 3.6 vs 3.2 mmol/L; ETD: -0.38 mmol/L [-0.70; -0.06];  $p=0.02$ . Rates of overall confirmed hypoglycaemia (PG  $<$  3.1 mmol/L or severe) were similar for the entire 2-yr period (1.72 [IDeg] vs 2.05 [IGlar] episodes/pt-yr; estimated rate ratio (ERR) IDeg/IGlar: 0.84 [0.68; 1.04];  $p=NS$ ). Severe hypoglycaemia was infrequent but significantly lower with IDeg (0.01 vs 0.02 episodes/pt-yr; ERR: 0.31 [0.11; 0.85];  $p=0.02$ ). Nocturnal confirmed hypoglycaemia rates were significantly 43% lower with IDeg (0.27 vs 0.46 episodes/pt-yr; ERR: 0.57 [0.40; 0.81];  $p<0.001$ ), indicating that treating 5.3 patients for 1 yr with IDeg will avoid 1 nocturnal confirmed hypoglycaemic episode. Mean daily basal insulin doses were similar at EOT (0.63 U/kg for IDeg and IGlar). Observed body weight increases (IDeg: 2.7 kg; IGlar: 2.4 kg) were similar, as were adverse event rates (3.6 vs 3.4 events/pt-yr).

**Conclusion:** When utilized as basal insulin in combination with OADs in T2DM over 2 yrs, degludec safely and effectively improves long-term glycaemic control with a lower risk of nocturnal and severe hypoglycaemia when compared to glargine.

*Clinical Trial Registration Number:* NCT01193309

*Supported by:* Novo Nordisk

## 921

**Insulin degludec is highly efficacious regardless of diabetes duration or body mass index: a cross-trial evaluation**L. Blonde<sup>1</sup>, L. Endahl<sup>2</sup>, N. Lassota<sup>2</sup>;<sup>1</sup>Ochsner Diabetes Research Unit, New Orleans, USA, <sup>2</sup>Novo Nordisk A/S, Søborg, Denmark.

**Background and aims:** Widely accepted treatment goals for antihyperglycaemic therapy include lowering HbA<sub>1c</sub> to levels of  $\leq$ 6.5% or  $<$ 7%. Not all patients are able to achieve these targets, for diverse reasons. We conducted a cross-trial evaluation to determine if certain baseline characteristics influence whether a patient achieves treatment goals with the new ultra-long-acting basal insulin, insulin degludec (IDeg).

**Materials and methods:** Three phase 3a trials, including previously insulin-naïve patients with type 2 diabetes (T2D) as well as patients with type 1 diabetes (T1D) on basal-bolus treatment, were used to assess whether certain baseline characteristics (HbA<sub>1c</sub>, known diabetes duration and BMI) differed between the entire IDeg-treated trial population and those individuals achieving HbA<sub>1c</sub>  $\leq$ 6.5% or  $<$ 7% with IDeg treatment. In trial A (trial 3668), a total of 228 patients with T2D previously insulin-naïve or receiving basal insulin treatment were given once-daily (OD) IDeg for 26 weeks. In trial B (trial 3582) and trial C (trial 3583), 744 and 472 previously insulin-treated patients with T2D and T1D, respectively, received IDeg OD for 52 weeks, with insulin aspart at mealtimes.

**Results:** The proportion of IDeg-treated patients achieving HbA<sub>1c</sub>  $\leq$ 6.5% or  $<$ 7% was 23% and 41%, respectively (trial A), 31% and 49%, respectively (trial B), and 24% and 40%, respectively (trial C). Mean baseline values (HbA<sub>1c</sub>, diabetes duration and BMI) varied across trials, reflecting the different patient populations being studied. In all three trials, patients achieving HbA<sub>1c</sub>  $\leq$ 6.5% or  $<$ 7% had a lower HbA<sub>1c</sub> at baseline compared with the entire population treated with IDeg (Table 1). Neither known duration of diabetes nor BMI were systematically different for patients achieving HbA<sub>1c</sub>  $\leq$ 6.5% or  $<$ 7% compared with the entire population.

**Conclusion:** This evaluation of patients with T2D or T1D suggests that neither diabetes duration nor BMI are factors that influence whether patients will achieve HbA<sub>1c</sub>  $\leq$ 6.5% or  $<$ 7.0% when initiating treatment with once-daily IDeg or switching to IDeg from another basal insulin. As expected, individuals achieving HbA<sub>1c</sub>  $\leq$ 6.5% or  $<$ 7% are characterised by a lower baseline HbA<sub>1c</sub>. Based on the above studies, the likelihood of a patient achieving HbA<sub>1c</sub> targets with once-daily IDeg is not influenced by diabetes duration or BMI at baseline, but by baseline HbA<sub>1c</sub>.



Table 1: Baseline HbA<sub>1c</sub>, but not diabetes duration or BMI, was associated with subjects reaching HbA<sub>1c</sub> targets across three randomised trials.

	HbA <sub>1c</sub> ≤6.5%	HbA <sub>1c</sub> <7%	All subjects
HbA <sub>1c</sub> (%)			
Trial A	7.8	7.9	8.4
Trial B	8.0	8.0	8.3
Trial C	6.8	7.0	7.7
Diabetes duration (years)			
Trial A	9.0	9.9	10.3
Trial B	14.0	13.9	13.6
Trial C	19.1	19.9	19.1
BMI (kg/m <sup>2</sup> )			
Trial A	28.4	29.0	29.4
Trial B	32.9	32.8	32.3
Trial C	26.7	26.5	26.3

Supported by: Novo Nordisk

## 922

### Pharmacokinetics (PK) of the novel, long-acting basal insulin LY2605541 in subjects with varying degrees of renal function

H. Linnebjerg, S.L. Choi, E.C.Q. Lam, K.F. Mace, T.S. Hodgson, V.P. Sinha; Eli Lilly and Company, Indianapolis, USA.

**Background and aims:** The basal insulin LY2605541 (LY) is PEGylated insulin lispro designed to have a large hydrodynamic size which delays insulin absorption and reduces clearance, resulting in prolonged duration of action. This Phase 1, multi-site, open-label study was designed to investigate the PK of LY in subjects with renal impairment.

**Materials and methods:** The PK of LY after a single subcutaneous dose (3 nmol/kg) was evaluated in 5 groups of subjects [Table]. Serial PK samples were collected up to 12 days post-dose. For subjects with end stage renal disease (ESRD), LY was given approximately 48 h before subjects resumed their normal dialysis schedule (2–4 hemodialysis sessions over the 12-day PK sampling period).

**Results:** The apparent clearance (CL/F) and half-life ( $t_{1/2}$ ) across the groups were not affected by renal function; the relationship between CL/F and the estimated creatinine clearance (CrCL) was not significant (slope=0.000863 [ $p=0.885$ ]). Dose-normalized  $C_{max}$  values ( $C_{max}/\text{Dose}$ ) in subjects with moderate and severe renal impairment were slightly lower compared to control subjects, but with similar overall LY serum exposure (dose-normalized  $AUC_{(0-\infty)}$  [ $AUC_{(0-\infty)}/\text{Dose}$ ]). Dialysis did not result in a significant LY elimination ( $\leq 25\%$ ) relative to the overall AUC in subjects with ESRD. LY was well tolerated in healthy subjects and those with renal impairment.

**Conclusion:** In conclusion, the PK properties of LY appear to be unaffected by renal impairment. Therefore no specific dose adjustment due to PK would be needed with increasing renal impairment.

	Estimated Creatinine Clearance (CrCL, mL/min)				
	Normal Function (>80)	Mild Impairment (51–80)	Moderate Impairment (30–50)	Severe Impairment (<30)	ESRD (Dialysis for >3 months)
Demographics [N or Mean (SD)]					
N (M/F)	12 (9/3)	8 (7/1)	8 (5/3)	9 (4/5)	9 (6/3)
Age (years)	45.5 (15.3)	68.9 (8.9)	61.0 (13.3)	61.8 (11.5)	44.4 (10.7)
Weight (kg)	81.3 (15.1)	84.5 (13.0)	73.7 (16.0)	66.7 (10.1)	79.9 (19.3)
Pharmacokinetic Parameters [Geometric Mean (% CV)]					
$AUC_{(0-\infty)}/\text{Dose}$ (pmol·hr/L/pmol)	0.352 (36)	0.452 (31)	0.429 (38)	0.424 (39)	0.323 (61)
CL/F (L/hr)	2.84 (36)	2.21 (31)	2.33 (38)	2.36 (39)	3.10 (61)
$t_{1/2}$ (hr)	34.9 (50)	37.2 (31)	43.7 (48)	42.4 (16)	45.7 (24)
$C_{max}/\text{Dose}$ (pmol/L/pmol)	0.00713 (78)	0.00863 (79)	0.00523 (37)	0.00532 (68)	0.00518 (155)

## 923

### Insulin degludec has similar pharmacokinetic properties in subjects with renal impairment and subjects with normal renal function

H.L. Haahr<sup>1</sup>, G. Arold<sup>2</sup>, S.G. Böttcher<sup>3</sup>, M. Thrane<sup>4</sup>, I. Kiss<sup>5</sup>;

<sup>1</sup>Department of Insulin Clinical Pharmacology, Novo Nordisk A/S, Søborg, Denmark, <sup>2</sup>PRA International GmbH, Berlin, Germany, <sup>3</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>4</sup>Novo Nordisk A/S, Copenhagen, Denmark, <sup>5</sup>St Imre Teaching Hospital, Budapest, Hungary.

**Background and aims:** Insulin degludec (IDeg) is a new-generation basal insulin forming soluble multi-hexamers upon subcutaneous injection, resulting in a flat and stable ultra-long action profile. This open-label, parallel-group trial investigated the pharmacokinetic (PK) properties of IDeg in subjects with different grades of renal impairment and subjects with normal renal function (NRF) following single doses of 0.4 U/kg IDeg. In addition, the influence of haemodialysis on clearance of IDeg was investigated in end-stage renal disease (ESRD) subjects by administration of two single doses of IDeg, one before and one just after haemodialysis.

**Materials and methods:** A total of 30 subjects (mean age: 65.6 years; females/males: 15/15; mean BMI: 28.4 kg/m<sup>2</sup>) were allocated to one of five renal function groups ( $n=6$  per group): NRF, mild, moderate, severe renal impairment or ESRD.

**Results:** PK profiles of IDeg were similar for subjects with normal and impaired renal function. Renal impairment had no statistically significant effect on total exposure ( $AUC_{0-120h,SD}$ ), maximum concentration ( $C_{max,SD}$ ) or apparent clearance ( $CL/F_{SD}$ ) [Table 1]. PK profiles of IDeg for subjects with ESRD were similar irrespective of whether subjects received haemodialysis or not. Haemodialysis did not affect CL/FSD, and no unaltered IDeg was detected in dialysate samples collected during dialysis from subjects with ESRD.

**Conclusion:** The ultra-long PK properties of IDeg are preserved in subjects with renal impairment; renal impairment did not result in differences in the PK properties of IDeg compared to subjects with NRF. Haemodialysis did not affect the clearance of IDeg.

#### PK endpoints vs. creatinine clearance

	$AUC_{IDeg,0-120h,SD}$	$C_{max,IDeg,SD}$	$CL/F_{IDeg,SD}$
Estimated slope	-0.138	-0.171	0.129
95% CI	[-0.390; 0.113]	[-0.415; 0.073]	[-0.120; 0.378]
Statistical significance	NS	NS	NS

Estimated slope: a measure of correlation between creatinine clearance and PK endpoint; CI: confidence interval; NS: non-significant ( $p \geq 0.05$ ).

Renal impairment was classified based on creatinine clearance ( $CL_{CR}$ ) estimated by the Cockcroft & Gault formula, and subjects were grouped as: Normal ( $CL_{CR} > 80$  mL/min), mild ( $CL_{CR} > 50$ – $\leq 80$  mL/min), moderate ( $CL_{CR} > 30$ – $\leq 50$  mL/min), severe ( $CL_{CR} \leq 30$  mL/min or end-stage renal disease (ESRD)).

Clinical Trial Registration Number: NCT01006057

Supported by: Novo Nordisk

## 924

### Ultra-long pharmacokinetic properties of insulin degludec in younger adults are preserved in geriatric subjects with type 1 diabetes

S. Korsatko<sup>1</sup>, S. Deller<sup>1</sup>, J. Mader<sup>1</sup>, K. Glettl<sup>1</sup>, G. Köhler<sup>1</sup>, G. Bock<sup>1</sup>, M. Urschitz<sup>1</sup>, M. Wolf<sup>1</sup>, H. Hastrup-Nielsen<sup>2</sup>, F. Søndergaard<sup>3</sup>, H.L. Haahr<sup>2</sup>, T.R. Pieber<sup>1</sup>;

<sup>1</sup>Medical University of Graz, Austria, <sup>2</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>3</sup>Novo Nordisk A/S, Aalborg, Denmark.

**Background and aims:** Insulin degludec (IDeg) is an ultra-long-acting basal insulin that forms soluble multi-hexamers upon subcutaneous injection resulting in a depot from which IDeg is continuously and slowly absorbed into the circulation leading to a flat and stable glucose-lowering effect. This study investigated the pharmacokinetic (PK) and pharmacodynamic (PD) properties of IDeg in geriatric ( $\geq 65$  years) versus younger adult (18–35 years) subjects with type 1 diabetes (C-Peptide  $< 0.3$  nmol/L).

**Materials and methods:** This was a randomised, double-blind, two-period cross-over, multiple-dose study with 6 days of once-daily administration of 0.4 U/kg IDeg or 0.4 U/kg insulin glargine. Data are shown for IDeg only. Fourteen geriatric (mean age: 67.8 years; baseline HbA<sub>1c</sub>: 7.7%; BMI: 26.2 kg/

m<sup>2</sup>) and 13 younger adult (mean age: 27.1 years; baseline HbA<sub>1c</sub>: 7.8%; BMI: 24.4 kg/m<sup>2</sup>) subjects participated.

**Results:** The mean IDeg concentration-time profile at steady state (SS) was similar in geriatric and younger adult subjects. There was no statistically significant difference in total exposure (AUC<sub>IDeg,tau,SS</sub>) or maximum concentration (C<sub>max,IDeg,SS</sub>) of IDeg between geriatric and younger adults (Table 1), mean ratio [95% CI] C<sub>max,SS</sub>: 1.02 [0.74; 1.39]. The estimated terminal half-life was 25 hours. There was no statistically significant difference between age groups in total glucose-lowering effect of IDeg (AUC<sub>GIR,tau,SS</sub>) (Table 1).

**Conclusions:** The ultra-long pharmacokinetic properties of IDeg in younger adults were preserved in geriatric subjects. Total exposure was similar and there were no differences in the glucose-lowering effect of IDeg between geriatric and younger adult subjects.

	PK: AUC IDeg,<tau>,SS (pmol*h/L)	PD: AUC GIR,<tau>,SS (mg/kg)
Geriatric (least square mean)	85673	1923
Younger adult (least square mean)	82727	2457
Geriatric vs. younger adult Mean ratio [95% CI]	1.04 [0.73; 1.47]	0.78 [0.47; 1.31]

Table 1: Pair-wise comparison of IDeg PK/PD in geriatric vs younger adult subjects with type 1 diabetes

Clinical Trial Registration Number: NCT00964418

Supported by: Novo Nordisk

## 925

### Insulin degludec provides similar pharmacokinetic and pharmacodynamic responses in Black, White and Hispanic/Latino patients with type 2 diabetes

M. Hompesch<sup>1</sup>, L. Morrow<sup>1</sup>, E. Watkins<sup>1</sup>, C. Roepstorff<sup>2</sup>, H.F. Thomsen<sup>3</sup>, H.L. Haahr<sup>2</sup>;

<sup>1</sup>Profil Institute for Clinical Research, Inc, Chula Vista, USA, <sup>2</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>3</sup>Novo Nordisk A/S, Aalborg, Denmark.

**Background and aims:** Insulin degludec (IDeg) is a new-generation basal insulin that forms soluble multi-hexamers upon s.c. injection, resulting in an ultra-long and consistent action profile. This randomised, double-blind, two-period, cross-over trial investigated the pharmacokinetic and pharmacodynamic properties of IDeg at steady-state (SS).

**Materials and methods:** The trial included 63 insulin-treated patients (26 females and 37 males) with type 2 diabetes of different race and/or ethnicity (18 Black, 23 White, 22 Hispanic/Latino; mean age: 52 years, BMI: 32.4 kg/m<sup>2</sup>, HbA<sub>1c</sub>: 8.2%). Patients completed two 6-day treatment periods with 0.6 U/kg-day of IDeg or insulin detemir (IDet). At the end of each treatment period, a 24-hour euglycaemic glucose clamp was performed (Biostat; clamp blood glucose level 5.0 mmol/L).

**Results:** IDeg resulted in a flat and stable glucose-lowering effect extending beyond 24 hours in all patients across race/ethnic groups. The effect was evenly distributed between the first and second 12 hours in all groups (AUC<sub>GIR,0-12h,SS</sub>/AUC<sub>GIR,<tau>,SS</sub> ranging between 0.48 and 0.54). The total glucose-lowering effect of IDeg at SS (AUC<sub>GIR,<tau>,SS</sub>) was 1940, 1722, and 2286 mg/kg in Black, White, and Hispanic/Latino subjects, respectively. Pair-wise comparisons were not significantly different (mean difference [95% CI]: Black vs. Hispanic/Latino -346 [-1088; 396]; Black vs. White 218 [-551; 986]; Hispanic/Latino vs. White 564 [-168; 1295] mg/kg). Pair-wise comparisons of total IDeg exposure (AUC<sub>IDeg,<tau>,SS</sub>) were also similar between the three groups with mean ratios [95% CI] of: Black vs. Hispanic/Latino 1.13 [0.95; 1.34], Black vs. White 1.10 [0.91; 1.31], and Hispanic/Latino vs. White 0.97 [0.82; 1.16] pmol-hour/L. There was no significant difference in the race/ethnicity pattern between IDeg and IDet for AUC<sub>GIR,<tau>,SS</sub>, p=0.85 or AUC<sub>ins,<tau>,SS</sub>, p=0.58. IDeg was well tolerated and no safety issues were identified.

**Conclusion:** IDeg resulted in similar pharmacokinetic and pharmacodynamic responses across race and ethnicity in patients with type 2 diabetes. Thus, the ultra-long pharmacological properties of IDeg are preserved across race and ethnic groups. While insulin doses must be adjusted on an individual basis, this trial supports the notion that differences in the response to IDeg should not be anticipated based on differences in race or ethnicity between patients.

Clinical Trial Registration Number: NCT01043510

Supported by: Novo Nordisk

## PS 075 Insulin delivery

### 926

#### Local tolerability of insulin degludec is comparable to insulin glargine: a meta-analysis of trials in type 1 and type 2 diabetes

L.F. Meneghini<sup>1</sup>, P.-M. Schumm-Draeger<sup>2</sup>, S. Harris<sup>3</sup>, M.-A. Gall<sup>4</sup>, N. Lassota<sup>4</sup>, J.S. Christiansen<sup>5</sup>;

<sup>1</sup>University of Miami Miller School of Medicine, Miami, USA, <sup>2</sup>Academic Teaching Hospital Munich Bogenhausen, Munich, Germany, <sup>3</sup>University of Western Ontario, London, Canada, <sup>4</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>5</sup>Aarhus University Hospital, Aarhus, Denmark.

**Background and aims:** Injection site reactions may occur following subcutaneous (s.c.) insulin administration. Insulin degludec (IDeg) is a new basal insulin that forms a soluble depot of multi-hexamers after s.c. injection.

**Materials and methods:** Utilising patient level data, we examined via meta-analysis whether the number of injection site reactions with IDeg was different from insulin glargine (IGlar) in patients with type 1 diabetes (T1D) or type 2 diabetes (T2D). The analysis included 6 randomised, open-label, controlled, treat-to-target, phase 3a trials of 26 or 52 weeks' duration with once-daily dosing of IDeg (n=3060) and IGlar (n=1198) in T1D (2 trials) and T2D (4 trials). There were more subjects treated with IDeg than IGlar due to a number of trials having a 3:1 randomisation. The number of injection site reactions was analysed with a negative binomial regression model.

**Results:** Although lower observed rates of injection site reactions were seen with IDeg compared to IGlar in both T1D and T2D (basal-only therapy), the differences were not statistically significant (Table). A similar proportion of patients (T1D+T2D) reported injection site reactions with IDeg (3.6%) and IGlar (3.5%). For both IDeg and IGlar, injection site reactions were most commonly reported as injection site haematoma, injection site reaction (unspecific) and injection site pain. Two injection site reactions for IGlar were classified as severe adverse events. Few patients (0.1%) with IDeg or IGlar withdrew from the trials due to injection site reactions (IDeg: injection site haematoma, injection site pain; IGlar: injection site reaction [unspecified]).

**Conclusion:** This meta-analysis demonstrates good local tolerability of IDeg compared to IGlar following s.c. injection.

#### Injection site reaction analysis outcomes

	Number of patients (n)		Observed rate per 100 exposure-years		Estimated rate ratio IDeg/IGlar	
	IDeg	IGlar	IDeg	IGlar	Rate ratio	95% CI
T1D+T2D (pooled)	3060	1198	6.8	7.0	0.86	[0.57; 1.30]
T1D <sup>a</sup>						
801			5.3	6.7	N/A	N/A
315						
T2D	2259	883	7.4	7.1	0.91	[0.56; 1.48]
T2D-BOT <sup>b</sup>	1506	632	8.7	9.4	0.72	[0.41; 1.27]

<sup>a</sup>Too few events to fit the statistical model; <sup>b</sup>Basal insulin-only therapy

Supported by: Novo Nordisk

### 927

#### Risk factors to predict insulin requirement for optimum control of blood glucose after glucocorticoid administration in patients with autoimmune diseases

H. Morita, T. Ikeda, I. Mori, Y. Kitada, K.-I. Taguchi, N. Takahashi, K. Fujioka, M. Yamauchi, K. Kajita, T. Ishizuka;  
Department of General Internal Medicine, Gifu University Graduate School of Medicine, Japan.

**Background and aims:** Glucocorticoid treatment often induces insulin resistance and hyperglycemia. High dose of glucocorticoid or the pulse therapy are initially required to treat severe disturbance of organs. It is important to predict in advance insulin requirement for optimum control of hyperglycemia induced by glucocorticoid administration for treatment of various autoimmune diseases. We aimed to investigate risk factors and their cutoff values to predict insulin requirement after glucocorticoid administration.

**Materials and methods:** We retrospectively evaluated patients with autoimmune diseases who admitted to our department between 2004 and 2011 and were treated with glucocorticoid. They were newly administered more than 5 mg/day of prednisolone and continued for at least 4 weeks. Patients who had already used insulin before initiation of glucocorticoid therapy and were administered GLP-1 analogue instead of insulin after glucocorticoid treatment were excluded.

**Results:** A total of 147 patients (mean age, 58±19 years) were analyzed in the present study. Insulin, oral antihyperglycemic agents and diet therapy alone were needed to improve hyperglycemia in 26, 24 and 97 patients, respectively. A multiple logistic regression analysis revealed that risk factors of insulin requirement after glucocorticoid administration were higher fasting plasma glucose, larger initial dose (mg/body weight) of prednisolone, higher HbA1c and male. Receiver operating characteristic (ROC) analysis showed that cutoff values of HbA1c, fasting plasma glucose and initial dose of prednisolone were 99 mg/dl, 0.74 mg/kg/day, 6.7%, respectively. Area under the curve of the ROC curve in fasting plasma glucose (0.85) was higher than in HbA1c (0.80) or initial dose of prednisolone (0.74).

**Conclusion:** More than 99 mg/dl of fasting plasma glucose before glucocorticoid administration is most valuable predicting factor for insulin requirement after glucocorticoid treatment.

## 928

### Night time glucose variability in obese type 2 diabetes patients treated with NPH insulin at bedtime may be related to the size of the needle

M. Pawlowski<sup>1</sup>, M. Rutkowska<sup>1</sup>, E. Szymanska-Garbacz<sup>1</sup>, M. Saryusz-Wolska<sup>1</sup>, J. Loba<sup>1</sup>, P. Grzelak<sup>2</sup>, L. Stefanczyk<sup>2</sup>, L. Czupryniak<sup>1</sup>; <sup>1</sup>Diabetologia, <sup>2</sup>Radiology, Medical University of Lodz, Poland.

**Background and aims:** In a large group of type 2 diabetes patients NPH insulin given at bedtime fails to control fasting blood glucose, even if a dose of the insulin is increased substantially. This problem is frequently encountered in obese subjects, in whom insulin absorption from subcutaneous tissue might be severely impaired and may depend on depth of the injection i.e. on the needle length. The effect of needle length on blood glucose control is poorly studied. The aim of this study was to assess the effect of using two various needle sizes on blood glucose excursions after NPH insulin dose administration.

**Materials and methods:** We studied 19 obese subjects (mean [±SD] age 62.3±5.0 years, 11 women, BMI 34.3±4.1 kg/m<sup>2</sup>), with long lasting type 2 diabetes (diabetes duration 14.3±4.2 years) and inadequate control of disease (HbA1c 8.6±1.1%). All subjects were treated with short acting insulin before meals and high dose of NPH insulin (46±11 IU) at bedtime. Subcutaneous fat tissue thickness assessed by ultrasound scanning was 2.1±0.6 cm. Glucose excursions were measured with the Continuous Glucose Monitoring System (CGMS; iPRO2, Medtronic, Minneapolis, USA) used consecutively for 5 days (4 nights), from Monday to Friday. For the first two days patients used 6 mm needle, and for the following two days they used 12.7 mm one. Insulin NPH was injected at the same site in the abdominal area. We compared fasting blood glucose (FBG), calculated average glucose (AG) and the total area under the curve (AUC) of glucose excursions during the night (0.00–6.00 am); glycemic variability coefficients were also calculated.

**Results:** Injecting NPH insulin with a 12.7 mm needle was associated with lower glycemia than when 6 mm needle was used: FBG was 134±16 vs 145±25 mg/dl (p=0.02), and AG 111.1±14.1 vs 133.8±32.7mg/dl (p=0.04), respectively. There was a trend for the AUC to be lower when 12.7 rather than 6 mm needle was used (7911±724 vs 9732±1641, p=0.04). Coefficient of variation was 11.1±6 when a short needle was used and 12.6±6.1 with a long one (p=0.37).

**Conclusion:** Poorly controlled obese type 2 diabetes patients may benefit from using longer needles for insulin NPH injections as it may result in the reduction of fasting blood glucose. Establishing whether this effect will be long lasting and eventually may lead to a significant HbA1c decrease requires further prospective studies.

Supported by: Medical University of Lodz, Medtronic Poland

## 929

### Efficacy of needle-free jet injection for the administration of rapid-acting insulin analogues is independent of the body-mass index

E.E.C. Engwerda<sup>1</sup>, E.J. Abbink<sup>2</sup>, C.J. Tack<sup>1</sup>, B.E. de Galan<sup>1</sup>; <sup>1</sup>General Internal Medicine 463, <sup>2</sup>Clinical Research Centre Nijmegen, Radboud University Nijmegen Medical Centre, Netherlands.

**Background and aims:** We previously showed that administration of rapid-acting insulin analogues by jet injection reduced the time to peak plasma insulin levels and the time to maximal insulin action by ~50%, when compared to conventional insulin injection by insulin pen. The current analysis was conducted to assess whether and to what extent body-mass index (BMI) affected the rate of insulin absorption and glucose-lowering action, when administered by jet injection or conventional insulin pen.

**Materials and methods:** Euglycaemic glucose clamps were performed in 18 healthy volunteers (BMI range, 18 to 28 kg/m<sup>2</sup>) after subcutaneous administration of 0.2 U/kg body weight of insulin aspart, either by jet injection or conventional pen, using a randomised, double-blind, double-dummy, crossover study design. The times to maximal plasma insulin level (T-INS<sub>max</sub>) and maximal glucose infusion rate (GIR) needed to maintain euglycemia (T-GIR<sub>max</sub>) were analysed in subgroups defined by BMI below the median of 23.6 kg/m<sup>2</sup> (BMI-low) or BMI above the median (BMI-high).

**Results:** After conventional insulin injection, T-INS<sub>max</sub> was 49±3 minutes in BMI-low versus 80±10 minutes in BMI-high (P=0.008), and T-GIR<sub>max</sub> was 77±10 in BMI-low versus 134±15 minutes in BMI-high (P=0.006). After jet injection, BMI-low and BMI-high subgroups did not differ with respect to T-INS<sub>max</sub> (29±4 versus 32±5 minutes, P=0.59) or T-GIR<sub>max</sub> (48±4 versus 54±4 minutes, P=0.31). In continuous analyses, BMI was significantly correlated to T-INS<sub>max</sub> (R=0.58, P=0.012) and T-GIR<sub>max</sub> (R=0.56, P=0.017) after conventional insulin administration, but not after jet injection (R=0.059 and 0.135, respectively, P both >0.5). As a consequence, the time-reducing benefit of using jet rather than conventional injection for insulin administration was greater in the BMI-high than in the BMI-low subgroup for both T-INS<sub>max</sub> (-48±9 versus -20±5 minutes, P=0.018) and T-GIR<sub>max</sub> (-80±16 versus -29±10 minutes, P=0.018).

**Conclusion:** BMI significantly affects the rate of absorption and action of aspart insulin when injected by conventional pen, but not when injected by jet injection. Therefore, using a jet injector for administration of rapid-acting insulin analogues may especially benefit subjects with higher BMI.

Clinical Trial Registration Number: NCT00983775

Supported by: European Pharma Group

## 930

### Clinical test of fuzzy logic controller (FLC) in a closed loop artificial pancreas

R. Mauseth<sup>1</sup>, D. Matheson<sup>1</sup>, R. Kircher<sup>1</sup>, J. Bollyky<sup>2</sup>, C. Greenbaum<sup>2</sup>;

<sup>1</sup>Dose Safety, Inc., Redmond, <sup>2</sup>Benaroya Research Institute, Seattle, USA.

**Background and aims:** To evaluate the effectiveness of a fully automated system using a FLC in a 24-hr clinical research center setting. We wished to determine whether this system could (1) correct elevated blood glucose (BG), (2) control the fasting state and diurnal variation, (3) control the glycemic response to a small meal (30 gms CHO) and (4) control the glycemic response to a larger meal (60 gms CHO). Prior to the study, in silico testing was performed and the dosing aggressiveness parameter was established. Hypoglycemia avoidance was the primary focus and blood sugar control was secondary for this study, our first fully automated, closed loop human trial.

**Materials and methods:** A FLC was used to direct insulin delivery to adult type 1 patients. We used the Artificial Pancreas System developed by UC Santa Barbara and Sansum Diabetes Research Institute, an OmniPod pump and Dexcom Seven Plus sensors. There was no pre-meal priming boluses, nurse or physician data entry or modifications. The controller algorithm was initialized using only the patient's Total Daily Insulin. The controller automatically commanded the OmniPod pump on a five-minute cycle time.

**Results:** 8 adult subjects (4 men, 4 women, age 18–35 years, mean 23.6, A1c 6.4–8.6%, mean 7.7%) completed the overnight portion of the study. Table 1 shows the endpoint values for the midnight to 8am period. Two patients (number 1 and 4) did not complete the entire study due to technical issues. Procedural adjustments were made and no further problems occurred with the final six patients.

**Conclusion:** These preliminary data indicate that a FLC, initialized using only the patient's total daily insulin, may provide effective closed loop regu-



lation of blood glucose and reduce the risk of nocturnal hypoglycemia in a clinical setting for adults with type 1 diabetes.

Table 1: Midnight - 8am Results

Endpoint	Midnight-8am
Max blood glucose (mg/dL)	195 mg/dL
Min blood glucose (mg/dL)	95 mg/dL
Mean blood glucose	135 mg/dL
Percent time < 70 mg/dL	0.5%
Percent time > 200 mg/dL	4.0%
Percent time 70-200 mg/dL	95.6%
Percent time > 180 mg/dL	7.5%
Percent time in 70-180 mg/dL	92.0%
Percent time > 144 mg/dL	36.0%
Percent time in 70-144 mg/dL	63.5%

Supported by: JDRF

## 931

### Lowering blood glucose with continuous intravenous insulin infusion may have effect on QT interval

E. Szymanska-Garbacz, M. Saryusz-Wolska, M. Pawlowski, J. Loba, L. Czupryniak;  
Internal Medicine and Diabetology Department, Medical University of Lodz, Poland.

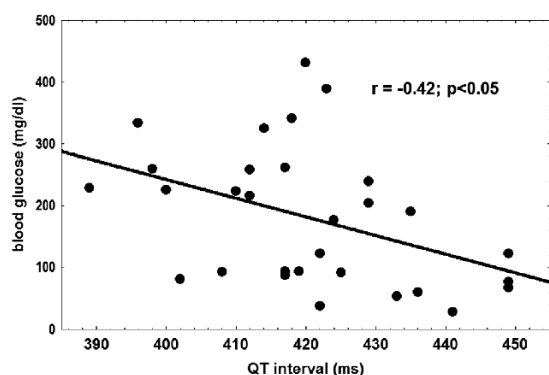
**Background and aims:** Poorly controlled type 1 diabetes patients are occasionally hospitalized and treated with continuous intravenous insulin infusion (CIVII), which is associated with a significant risk of hypoglycemia, an acknowledged risk factor for cardiac arrhythmias. The aim of the study was to assess heart rhythm during CIVII.

**Materials and methods:** The study group comprised 15 type 1 diabetes subjects (mean [±SD] age 32.6±10.7 years, diabetes duration 7.8±6.5 years, BMI 23.1±2.2 kg/m<sup>2</sup>, HbA1c 10.0±2.3%, pH 7.419±0.08, K<sup>+</sup> 4.34±0.39 mmol/l) without any heart disease or macrovascular complications or autonomic neuropathy, using no QT-affecting medications. CIVII consisted of basal insulin infusion and three 90-min mealtime boluses per day. Capillary blood glucose was measured every 60-90 min, and the CIVII rate was adjusted accordingly so as to achieve near normoglycemia. In all subjects 24-hour ECG monitoring was performed during the second day of CIVII.

**Results:** Mean blood glucose during CIVII was 174±51 mg/dl (range 28-432 mg/dl). No clinically significant abnormalities in ECG monitoring were noted. Mean QT interval was 422±14 ms. However, in the whole group there was a statistically significant correlation between QT interval during minimum and maximum blood glucose and these blood glucose values ( $r=-0.42$ ,  $p<0.05$ , Fig. 1). In addition, more insulin resistant subjects showed tendency to have longer QT interval ( $r=0.25$ ;  $p=0.06$ ).

**Conclusion:** In conclusion, using CIVII in otherwise healthy type 1 diabetes subjects may affect electrophysiological processes in the myocardium. This mode of treatment should be used with caution even in patients without cardiovascular disease.

Fig. 1. The correlation between QT interval during minimum and maximum blood glucose and these blood glucose values during CIVII (two blood glucose values per each patient).



Supported by: Medical University of Lodz

## 932

### Effect of intraperitoneal insulin administration on IGF-1 concentrations in type 1 diabetes

P.R. van Dijk<sup>1</sup>, S.J.J. Logtenberg<sup>2</sup>, N. Kleefstra<sup>1,2</sup>, K.H. Groenier<sup>1</sup>, H.J.G. Bilo<sup>1,2</sup>, H.J. Arnqvist<sup>3</sup>;

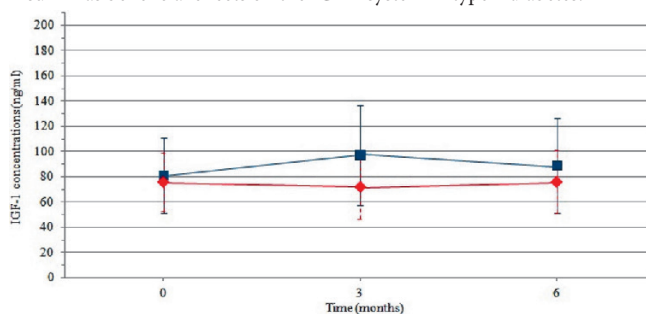
<sup>1</sup>Diabetes centre, Isala Clinics, Zwolle, Netherlands, <sup>2</sup>Internal Medicine, University Medical Center Groningen, Netherlands, <sup>3</sup>Diabetes Research Centre, Linköping University, Sweden.

**Background and aims:** Insulin-like growth factor-1 (IGF-1) and insulin genes are part of the same family. In patients with type 1 diabetes, low IGF-1 concentrations and high levels of IGF binding protein-1 (IGFBP-1) have been reported. This may affect atherosclerotic processes and diabetic vascular lesions. It has been suggested that the low circulating IGF-1 and high IGFBP-1 is partly due to low insulin levels in the portal vein. The aim of this study is to investigate the effects of intraperitoneal (IP) administered insulin as compared to subcutaneous (SC) insulin on IGF-1 and IGFBP-1 levels.

**Materials and methods:** Open-label, randomized controlled cross-over trial. After a 3-month qualification phase, patients were randomized to receive either 6 months continuous intraperitoneal insulin infusion (CIPII) through an implantable pump (MIP 2007C, Medtronic) followed by 6 months SC insulin or vice versa with a washout phase in between. General linear models were used to test for differences in HbA1c values, taking the order of the two treatments into account. Due to missing values, linear mixed models were used for IGF-1 and IGFBP-1. Correlation between differences in IGF-1 and total daily insulin dose was calculated using Spearman's rho.

**Results:** Twenty-three patients (43.4% male) were included with a mean (±SD) age of 43.1 ± 12.0 years, diabetes duration of 22.1 ± 10.9 years and HbA1c of 8.6 ± 1.1 %. Mean IGF-1 concentrations at the start of the SC and IP insulin phase were available for 21 and 22 patients, respectively; 75.8 (23.3) ng/ml and 81.1 (30.1) ng/ml. After randomization, groups were comparable regarding clinical characteristics. After 3 and 6 months the IGF-1 levels with SC insulin were 72.1 (25.5) ng/ml and 76.0 (25.1) ng/ml compared to 97.2 (39.9) ng/ml and 89.0 (37.5) ng/ml with IP insulin. The change in mean IGF-1 concentration during CIPII was 12.3 ng/ml (95% confidence interval (CI) 1.4, 23.2 ng/ml;  $p=0.03$ ) and 0.7 ng/ml (95% CI -10.0, 11.5 ng/ml;  $p=0.88$ ) during SC insulin. Taking the effect of treatment order into account, the estimated increase of IGF-1 was 11.6 ng/ml (95% CI -3.3, 26.5 ng/ml;  $p=0.12$ ) with CIPII compared to SC insulin. In addition, the IGFBP-1 concentrations decreased with -138.4 ng/ml (95% CI -206.7, -70.1 ng/ml;  $p<0.001$ ) with CIPII. HbA1c improved -0.76% (95% CI -1.41, -0.11) in favor of IP insulin. There was a non-significant negative association between the mean difference in insulin dose and IGF1 level during IP treatment ( $r=-0.038$ ,  $p=0.87$ ).

**Conclusion:** During CIPII treatment there was a decrease in IGFBP-1 and a non-significant increase in IGF-1 compared to the SC insulin phase. Taken together, the findings in the current study support the hypothesis, but do not provide definite answers to the question whether IP insulin compared to SC insulin has beneficial effects on the IGF-1 system in type 1 diabetes.



Clinical Trial Registration Number: NCT00286962

## 933

**Comparison of glycaemic response to typical foods of the mediterranean diet using different types of insulin bolus in patients with type 1 diabetes treated with insulin pump**S. Murillo<sup>1</sup>, L. Brugnara<sup>1</sup>, E. Roura<sup>2</sup>, A. Novials<sup>1</sup>;<sup>1</sup>CIBERDEM - IDIBAPS, Hospital Clinic, Barcelona, <sup>2</sup>ALICIA foundation, St Fruitós de Bages, Spain.

**Background and aims:** The glycaemic index of foods explains the effects of certain foods on blood glucose during the 2 hours after ingestion. However, some foods have a delayed absorption and, in these cases, the application of extended or multi-wave insulin boluses could be more effective in controlling postprandial glycaemia than traditional normal boluses. The aim of this study was to analyze the postprandial blood glucose effects after the ingestion of 4 typical menus in the Mediterranean diet, comparing the results obtained by applying 3 types of preprandial insulin boluses.

**Materials and methods:** 36 individuals with T1D over 18 years of age, and using insulin pump therapy, were included in the study. The dinner menus included ham and cheese pizza, seafood paella, cured ham sandwich and barley casserole. All menus contained the same amount of carbohydrates (60.5±1.2g) and similar total energy values (664.7±58.9kcal). For three non-consecutive days, individuals included in the study had the same menu for dinner, using the same dose of insulin as a bolus (previously calculated using each subject's own particular ratio insulin / carbohydrates ratio), but applying a Normal bolus, an Extended bolus to 4 hours or a Multi-wave bolus (50% normal and 50% extended to 4 hours) on each day of the study. Postprandial blood glucose was monitored by a continuous glucose sensor (DexCom 7Plus) and we monitored levels of physical activity by an activity sensor (Armband of Sensewear). We evaluated the area under the curve (AUC), expressed as mg/dlx8hours, and obtained within 8 hours after the dinner, for the same menu. Statistical analysis was performed using Student's *t*-test. The *p*-values < 0.05 were considered to be significant.

**Results:** Pizza: AUC values using Normal Bolus (390.5±162.2) were lower than when using the Extended (539.3±181.4;ns) or Multi-wave (460.7±217.7;ns) bolus. There was an increased number of hypoglycaemic events using the Normal bolus (33%) than with a Multi-wave (11%) or Expanded (11%) bolus. In a later study, we performed a test with 9 individuals who ate only pizza dough (without cheese or ham) using a Normal bolus. AUC values were lower (172.0±57.7) in comparison with full pizza (390.5±162.2; *p*<0.05). Seafood paella: Normal bolus produces less AUC (198.5±140.9) than Extended (469.4±97.8; *p*<0.05) or Multi-wave (348.7±152.0;ns) bolus. Sandwich: Normal Bolus has a lower AUC (181.6±107.1) than the Extended bolus (495.0±183.3, *p*<0.05) or the Multi-wave bolus (393.0±195.0, *p*<0.05). Barley casserole: Normal bolus caused hypoglycaemia in 6 of the 9 subjects studied. Extended bolus had a higher but, not significant, AUC (317.7±198.8) than Multi-wave bolus (210.0±16.9;ns).

**Conclusion:** Normal bolus is effective for those menus based on high-glycaemic index foods, such as sandwiches or seafood paella. For the paella, having a long cooking time (15 minutes), behaves as fast-food absorption, despite the moderate glycaemic index of rice. In contrast, Multi-wave bolus seems advisable for a slow-digesting food, like pizza or barley, since Normal bolus increases the frequency of hypoglycaemic events.

Supported by: ACD Grant 2010

## PS 076 Initiation of insulin therapy

## 934

**The effect of short-term continuous intravenous insulin infusion on long-term metabolic control in obese patients with type 2 diabetes**

M. Rutkowska, M. Pawlowski, J. Loba, L. Czupryniak;

Internal Medicine and Diabetology Department, Medical University of Lodz, Poland.

**Background and aims:** In a large group of obese type 2 diabetes achieving good metabolic control is extremely difficult, despite using all treatment options available in the outpatient setting (i.e. intensive education, intensive high-dose insulin treatment combined with oral agents administration). These patients may be treated with continuous intravenous insulin infusion (CIVII) in hospital as this mode of treatment improves blood glucose rapidly and is believed to counteract glucose toxicity. However, the long term effect of this procedure on metabolic control of diabetes is unknown. We conducted the study to assess the effect of short term CIVII on long term glucose control in obese subjects with type 2 diabetes.

**Materials and methods:** The study group comprised 36 type 2 diabetes patients treated with insulin (23 women and 13 men, mean age [±SD] 59.9 ± 7.7 years, diabetes duration 6.3 ± 3.0 years, body weight 92.9 ± 19.1 kg, BMI 33.5 ± 5.8 kg/m<sup>2</sup>, HbA1c 9.7 ± 1.8 %). In all subjects body weight, BMI, waist-to-hip ratio (WHR), blood pressure, fasting plasma triglycerides, total cholesterol, LDL- and HDL-cholesterol, and HbA1c levels were measured before and 6 months after CIVII. CIVII was applied for at least 72 hours, it consisted of basal insulin infusion and three 90-min insulin boluses per day administered at the meal time. Capillary blood glucose was measured every 90-120 min throughout the day and night. Hypoglycemia was diagnosed if capillary blood glucose was <60 mg/dl or when CIVII was stopped due to the symptoms of hypoglycemia. During the study period the subjects who used oral antidiabetic medication maintained it at stable doses. The controls were 24 type 2 diabetes patients (mean age 61.3 ± 7.2 years, BMI 32.8 ± 5.1 kg/m<sup>2</sup>, HbA1c 9.4 ± 1.6 %), who were subject to standard outpatient care and in whom the same parameters as in the study group were examined at baseline and after 6-month period.

**Results:** In obese type 2 patients 6 months after CIVII treatment HbA1c decreased significantly to 8.8 ± 1.6% (*p*<0.05), however no improvement in body weight, WHR, blood pressure or plasma lipid parameters was noted. Shortly after CIVII daily insulin dose was significantly reduced (from 64.5 ± 24.6 at baseline to 50.7 ± 10.8 IU/day on discharge from the hospital, *p*<0.05), yet 6 months later it was similar to the baseline insulin requirement. In the control subjects no statistically significant changes in analyzed parameters were found during the study period.

**Conclusion:** In patients with type 2 diabetes with poor metabolic control CIVII results in significant improvement of long-term glucose control, without exerting effect on other metabolic parameters.

## 935

**Comparison of three titration algorithms for the initiation of basal insulin in patients with type 2 diabetes mellitus**L. Aurand<sup>1</sup>, G. Dailey<sup>2</sup>, J. Stewart<sup>3</sup>, B. Ameer<sup>4</sup>, R. Zhou<sup>5</sup>;<sup>1</sup>Sanofi-aventis U.S., Bridgewater, USA, <sup>2</sup>Scripps Clinic, La Jolla, USA,<sup>3</sup>Sanofi-Aventis Canada, Quebec, Canada, <sup>4</sup>Robert Wood Johnson Medical School, New Brunswick, USA, <sup>5</sup>Medpace, Cincinnati, USA.

**Background and aims:** On initiation of basal insulin therapy in patients with type 2 diabetes mellitus (T2DM), the dose is titrated until target fasting plasma glucose (FPG) is achieved. Many different basal insulin titration algorithms have been studied and it may be difficult for health care providers to choose one. This pooled analysis of patient-level data compared endpoints from studies using different treatment algorithms for the initiation and intensification of insulin glargine, in insulin-naïve patients with T2DM.

**Materials and methods:** Data were pooled from 8 randomized controlled trials that added insulin glargine to oral antidiabetic drugs (OADs) at a 10 U starting dose, using 3 different treatment algorithms. Differences in the baseline characteristics between algorithms were analyzed by *t*-test or  $\chi^2$  test where appropriate. Change from baseline endpoint variables were analyzed using a mixed model with algorithm as a factor and corresponding baseline measurement as covariate.

**Results:** Algorithm 1 ( $n = 163$ ; mean age 57 years; 66% men) required 1 U daily when FPG was above target; algorithm 2 ( $n = 117$ ; mean age 59 years; 62% men) required 2 U every 3 days when FPG was above target; and algorithm 3 ( $n = 1100$ ; mean age 57 years; 54% men) used a treat-to-target approach, generally increasing 2–8 U weekly based on 2-day mean FPG levels. At baseline, there were differences in FPG and  $HbA_{1c}$  levels between the cohorts (Table). After adjusting for the baseline measurements, algorithm 2 had a significantly greater change in  $HbA_{1c}$  than did 1 (Table). Algorithm 3 tended towards more confirmed hypoglycemia, and in all groups the incidence of severe hypoglycemia was low, ranging 0–1.5% (Table). However, comparison of severe hypoglycemia between groups was limited due to few events. Final insulin doses were 0.43 U/kg, 0.60 U/kg, and 0.44 U/kg for algorithms 1, 2, and 3, respectively; with significantly higher doses for algorithm 2 vs 1 and 3, although when insulin dose at endpoint was corrected for  $HbA_{1c}$  change, doses were only significantly different for algorithm 2 vs 3. Baseline OAD therapy use differed across groups; those receiving metformin and a sulfonylurea (SU) made up 51%, 70% and 71% of algorithms 1, 2 and 3 respectively. When patients taking only metformin plus sulfonylurea at baseline were analyzed the results were comparable to those found in the whole population (Table).

**Conclusion:** These data suggest that simpler titration algorithms (1 and 2) achieved similar glycemic control vs more complex algorithms (3), with less confirmed hypoglycemia. These data may assist the choice of algorithm for initiation and intensification of basal insulin in patients with T2DM, but need validation with a randomized controlled trial.

Table: Baseline values and outcomes for 3 titration algorithms.

	Total Population ( $n=1380$ )			Metformin and SU ( $n=948$ )		
	Algo- rithm 1	Algo- rithm 2	Algo- rithm 3	Algo- rithm 1	Algo- rithm 2	Algo- rithm 3
Baseline $HbA_{1c}$ , %	8.6*	8.8	8.8*	8.5	8.8	8.8
Week 24 $HbA_{1c}$ , %	7.1	6.9	7.0	7.1	7.0	7.1
Achieving $HbA_{1c} < 7.0\%$ , %	53	61	52	52	54	50
Change in $HbA_{1c}$ , %	−1.54 <sup>†</sup>	−1.91 <sup>†</sup>	−1.81	−1.43	−1.82	−1.70
Baseline FPG, mg/dL	192 <sup>†</sup>	223 <sup>†‡</sup>	198 <sup>‡</sup>	190 <sup>†</sup>	227 <sup>†‡</sup>	194 <sup>‡</sup>
Week 24 FPG, mg/dL	120	126	121	122	129	121
Confirmed hypoglycemia < 56 mg/dL, n/N (%)	38/163 (23.3)	22/117 (18.8)	382/1100 (34.7)	25/83 (30.1)	19/84 (22.6)	318/781 (40.7)
Confirmed hypoglycemia < 70 mg/dL, n/N (%)	68/163 (41.7)	31/117 (26.5)	560/1100 (50.9)	43/83 (51.8)	24/84 (28.6)	449/781 (57.5)
Confirmed nocturnal hypoglycemia < 56 mg/dL, n/N (%)	12/163 (7.4)	7/117 (6.0)	214/1100 (19.5)	11/83 (13.3)	6/84 (7.1)	183/781 (23.4)

\* $P < 0.05$ , algorithm 1 vs algorithm 3; <sup>†</sup> $P < 0.05$ , algorithm 1 vs algorithm 2; and <sup>‡</sup> $P < 0.05$ , algorithm 2 vs algorithm 3.  
FPG, fasting plasma glucose.

Supported by: Study funding and editorial support provided by Sanofi-aventis U.S.

## 936

### Real-world comparative outcomes study in US patients with type 2 diabetes initiating basal insulin analogues

K.L. Davis<sup>1</sup>, J.L. Meyers<sup>1</sup>, M. Tangirala<sup>2</sup>, W. Wei<sup>2</sup>;

<sup>1</sup>RTI Health Solutions, Research Triangle Park, <sup>2</sup>Sanofi-Aventis U.S., Bridgewater, USA.

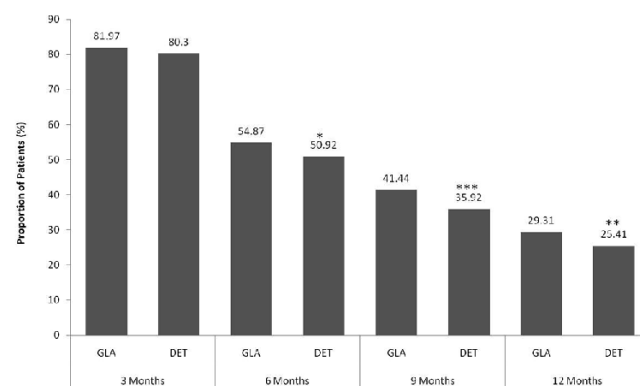
**Background and aims:** Currently, there is a paucity of real-world comparative effectiveness data among patients with type 2 diabetes mellitus (T2DM) initiating basal insulin analogs. Using a database of anonymized electronic medical records (EMR), this study compared outcomes among real-world US T2DM patients initiating insulin therapy with either insulin glargine (GLA) or insulin detemir (DET).

**Materials and methods:** Using the General Electric Centricity EMR database, a retrospective comparative analysis was conducted among T2DM patients who were  $\geq 18$  years old, initiated GLA or DET between January 1, 2006, and December 31, 2010, had EMR activity for  $\geq 6$  months prior to (baseline) and  $\geq 12$  months after (follow-up) GLA or DET initiation, and were previously or currently treated with only oral antidiabetic drugs (OADs) and/or a glucagon-like peptide-1 (GLP-1) analogue. To limit selection bias, each DET patient was matched with up to 5 GLA patients based on their baseline demographic and clinical characteristics. Study endpoints included

treatment persistence,  $HbA_{1c}$ , body weight, BMI, and chart-recorded hypoglycemia rate during the 12 month follow-up.

**Results:** A total of 6,620 (GLA: 5,473, DET: 1,147) patients were matched and analysed from 11,727 (GLA: 10,580, DET: 1,147) eligible patients. Patients were well balanced on the observed baseline characteristics (mean, GLA vs DET: male [%], 49.0 vs 48.5; age [years] 57.8 vs 57.4;  $HbA_{1c}$  [%], 9.4 vs 9.4; body weight [kg], 102.1 vs 102.6; BMI [kg/m<sup>2</sup>] 35.7 vs 35.9). Other well balanced baseline characteristics were geography, payer type, provider specialty, year of insulin initiation, prior chronic medication use and comorbidities. During the 1-year follow-up, significantly fewer patients were still taking DET at the end of 6, 9, and 12 months post-initiation (Figure). Prevalence of hypoglycemia was similar during the 1-year follow-up (2.87 vs 3.23%,  $P = 0.5143$ ). At the end of follow-up, the GLA cohort had significantly lower  $HbA_{1c}$  than the DET cohort (8.24 vs 8.37%,  $P = 0.0353$ ), the change in  $HbA_{1c}$  from baseline was also greater in the GLA cohort (−1.11 vs −0.96%,  $P = 0.0479$ ). There was no significant difference in weight (102.8 vs 102.8 kg,  $P = 0.9538$ ) or weight change from baseline (0.65 vs 0.40 kg,  $P = 0.3116$ ). BMI was also similar at the end of 1 year follow-up (35.92 vs 36.03 kg/m<sup>2</sup>,  $P = 0.6848$ ).

**Conclusion:** This EMR study of almost 7,000 matched US T2DM patients initiating basal insulin analogs suggested that, in the real-world setting, initiating GLA, as compared to DET, may be associated with greater persistence and better glycemic outcomes with similar hypoglycemia incidence and weight/BMI change.



Supported by: Study funding and editorial support provided by Sanofi-aventis U.S.

## 937

### The effects of early basal insulin treatment on inflammation and endothelium-related biomarkers in patients with type 2 diabetes

U. Ünlütürk<sup>1</sup>, A.S. Kesikli<sup>2</sup>, C. Ates<sup>3</sup>, G. Günaydin<sup>2</sup>, A.R. Uysal<sup>1</sup>, R. Emral<sup>1</sup>;

<sup>1</sup>Endocrinology and Metabolism, Ankara University School of Medicine, <sup>2</sup>Basic Oncology, Hacettepe University Institute of Oncology, <sup>3</sup>Biostatistics, Ankara University School of Medicine, Turkey.

**Background and aims:** In type 2 diabetes mellitus (T2DM), there is a lack of comparison of different treatment alternatives, especially in terms of pleiotropic effects such as the effects on endothelial functions, coagulation status and inflammation. This study was mainly prepared to analyze the effects of early basal insulin versus sulfonylurea treatment on endothelial injury, coagulation status and inflammation-related biomarkers in patients with T2DM.

**Materials and methods:** A total of 43 patients with T2DM, 16 in the insulin Detemir treatment arm and 27 in the gliclazide-MR arm were included. The study protocol is summarized in Figure 1. Plasma levels of soluble markers of endothelial activation or damage, such as intercellular cell adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1, E-selectin and P-selectin; markers of inflammation like monocyte chemoattractant protein-1 and interleukin-6, and markers to determine the tendency towards coagulation, like tissue plasminogen activator and plasminogen activator inhibitor-1 levels were investigated using commercially available ELISA kits.

**Results:** Both treatment protocols were equally effective in controlling blood glucose. sICAM-1 levels were observed to decrease significantly between the basal and 6<sup>th</sup> month follow-up measurements in the early insulin treatment arm. Moreover, sE-selectin levels were demonstrated to decrease between the basal and 3<sup>rd</sup> month follow-up measurements only in the insulin Detemir arm. Although sP-selectin levels were demonstrated to increase sig-



nificantly in both patient groups, the increment in the insulin Detemir arm was significant only between the 3<sup>rd</sup> and 6<sup>th</sup> month follow-up measurements, showing the effect of the addition of sulfonylurea to the treatment protocol. Levels of other markers were indifferent between the two treatment groups. **Conclusion:** These results suggest that early basal insulin treatment might be a promising approach in preventing late complications associated with T2DM, through inhibiting endothelial activation and damage.

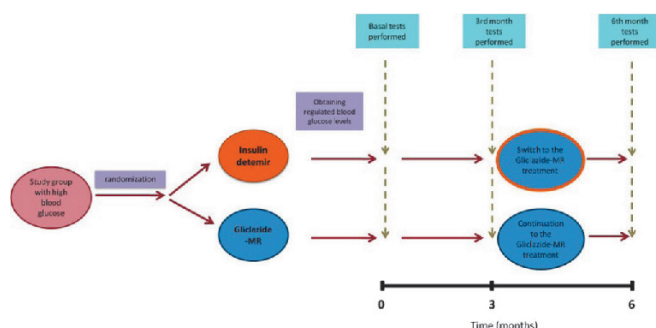


Figure 1: The summary of the work plan of the study

Clinical Trial Registration Number: NCT01420692

Supported by: TUBITAK

## 938

### Reduction in HbA<sub>1c</sub> and hypoglycaemia rates with insulin glargine vs NPH in treatment-naïve patients with type 2 diabetes stratified by BMI: pooled analysis of 6 clinical trials

G.B. Bolli<sup>1</sup>, F. Porcellati<sup>1</sup>, J. Lin<sup>2</sup>, A. Cali<sup>3</sup>, E. Wang<sup>4</sup>, M.-P. Dain<sup>3</sup>, P. Lucidi<sup>1</sup>, C.G. Fanelli<sup>1</sup>

<sup>1</sup>University of Perugia, Italy, <sup>2</sup>Novosys Health, Flemington, USA, <sup>3</sup>Sanofi, Paris, France, <sup>4</sup>Sanofi, Bridgewater, USA.

**Background and aims:** Insulin glargine is as efficacious as NPH insulin but is associated with lower incidence of hypoglycaemia. The aims of this pooled analysis were to compare efficacy and safety of glargine and NPH in treatment-naïve patients with type 2 diabetes (T2DM). Additional analysis was conducted to determine HbA<sub>1c</sub> reduction and incidence of severe and severe nocturnal hypoglycaemia based on BMI strata (< 30 kg/m<sup>2</sup> and ≥ 30 kg/m<sup>2</sup>) for patients using insulin glargine or NPH.

**Materials and methods:** Intent-to-treat populations were pooled from 6 randomised clinical trials of subjects with T2DM begun on insulin for 24 to 36 weeks. HbA<sub>1c</sub> and BMI were measured at baseline and endpoint. Severe and severe nocturnal hypoglycaemia were recorded.

**Results:** A total of 2600 subjects received either glargine (n = 1385) or NPH (n = 1215). There were no differences in baseline characteristics between groups, and 35% vs 34% had BMI ≥ 30 kg/m<sup>2</sup> in the glargine and NPH groups, respectively. At endpoint, in the overall analysis, HbA<sub>1c</sub> levels fell from mean (SD) 9.0 (1.0) % in both to 7.6 (1.2) % in the glargine group and 7.7 (1.2) % in the NPH group. The change from baseline was similar in both groups (-1.3 [1.2] %). In patients with BMI < 30 kg/m<sup>2</sup>, glargine use was associated with a significant reduction in HbA<sub>1c</sub> compared with NPH (-1.29 vs -1.14%, *P* = 0.008). The reduction in HbA<sub>1c</sub> was similar for both treatments in patients with BMI ≥ 30 kg/m<sup>2</sup>. Fewer subjects on glargine compared with NPH experienced severe hypoglycaemia, both overall and in the BMI < 30 kg/m<sup>2</sup> subgroup (Overall: 2.0% vs 3.2%; *P* = 0.04; BMI < 30 kg/m<sup>2</sup>: 2.2% vs 3.9%, *P* = 0.04, respectively). Treatment with glargine was associated with lower incidence of severe nocturnal hypoglycaemia compared with NPH overall and in 2 BMI subgroups (Overall: 0.7% vs 2.1%; *P* = 0.002; BMI < 30 kg/m<sup>2</sup>: 1.0% vs 2.4%, *P* = 0.02; BMI ≥ 30 kg/m<sup>2</sup>: 0.2% vs 1.7%, *P* = 0.01, respectively).

**Conclusion:** This pooled analysis shows that in patients with BMI < 30 kg/m<sup>2</sup> glargine is more efficacious and there is less severe hypoglycaemia than with NPH. The incidence of severe nocturnal hypoglycaemia was lower for glargine than NPH irrespective of BMI.

	Body Mass Index				Overall	
	< 30 kg/m <sup>2</sup>		≥ 30 kg/m <sup>2</sup>			
	Glargine	NPH	Glargine	NPH	Glargine	NPH
Δ HbA <sub>1c</sub> , Mean (SD), %	-1.3 (1.2) <sup>a</sup>	-1.1 (1.2)	-1.4 (1.2) <sup>d</sup>	-1.5 (1.1)	-1.3 (1.2) <sup>a</sup>	-1.3 (1.2)
Severe hypoglycaemia, %	2.2 <sup>b</sup>	3.9	1.4 <sup>d</sup>	1.9	2.0 <sup>b</sup>	3.2
Severe nocturnal hypoglycaemia, %	1.0 <sup>c</sup>	2.4	0.2 <sup>e</sup>	1.7	0.7 <sup>f</sup>	2.1

<sup>a</sup>*P* = 0.008; <sup>b</sup>*P* = 0.04; <sup>c</sup>*P* = 0.03; <sup>d</sup>*P* = NS; <sup>e</sup>*P* = 0.02; <sup>f</sup>*P* = 0.002 vs NPH

Supported by: Sanofi

## 939

### Weight change upon initiation of insulin detemir treatment with or without dietary intervention

P.A. Hollander<sup>1</sup>, M. Piletić<sup>2</sup>, H.J. Silver<sup>3</sup>, H. Andersen<sup>4</sup>, L. Conradsen Hiort<sup>4</sup>, K. Niswender<sup>3</sup>

<sup>1</sup>Baylor Endocrine Center, Baylor University Medical Center, Dallas, USA,

<sup>2</sup>General Hospital Novo Mesto, Novo Mesto, Slovenia, <sup>3</sup>Vanderbilt University School of Medicine, Nashville, USA, <sup>4</sup>Novo Nordisk, Søborg, Denmark.

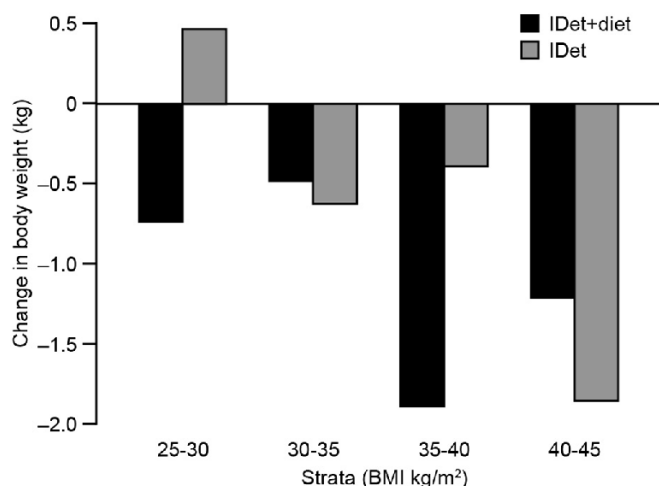
**Background and aims:** Weight gain upon initiation of insulin therapy is counter to clinical goals in diabetes management. The aim of this trial was to determine the impact of a dietary intervention on weight change from baseline when initiating insulin detemir (IDet) in insulin-naïve type 2 diabetes subjects.

**Materials and methods:** In all, 611 insulin-naïve type 2 diabetes subjects were included in a randomised, stratified, controlled, parallel, open-labelled multinational treat-to-target trial. The treatment period was 26 weeks. Subjects received IDet once daily with metformin and were randomised (1:1) to either dietary intervention (IDet+Diet) or no dietary intervention (IDet). Subjects were stratified according to one of 4 BMI strata: 25.0–29.9, 30.0–34.9, 35.0–39.9 and 40.0–45.0 kg/m<sup>2</sup>. Baseline (mean/SD) body weight and HbA<sub>1c</sub> were comparable for subjects randomised to the IDet+Diet group (weight 96.4/18.2 kg; HbA<sub>1c</sub> 8.0/0.7 %) and the IDet group (weight 97/20.4 kg; HbA<sub>1c</sub> 7.9/0.6 %). Subjects in the IDet+Diet group received 3 face-to-face meetings and 3 phone consultations by a certified dietician. Subjects in the IDet group received only basic dietary advice at baseline. Differences between the groups in changes from baseline in body weight, HbA<sub>1c</sub>, fasting plasma glucose (FPG) and hypoglycaemic episodes were analysed after 26 weeks.

**Results:** No differences in demographic or subject characteristics were seen between the groups at baseline. After 26 weeks the estimated mean weight loss (SEM) was 1.05 kg (0.23) (IDet+Diet) and 0.56 kg (0.23) (IDet); the difference was non-significant (*p* = 0.132). In both groups subjects in the highest BMI strata obtained a greater weight reduction compared to subjects in the lowest BMI strata (Figure 1). Both groups obtained a similar improvement in glycaemic control; estimated mean change (SE) in HbA<sub>1c</sub> after 26 weeks was -0.93 % (0.05) (IDet+Diet) and -0.80 % (0.05) (IDet); the difference was NS (*p* = 0.053). Estimated mean change (SE) in FPG was -3.00 mmol/l (0.12) (IDet+Diet) and -2.93 mmol/l (0.12) (IDet). No observed difference in caloric intake was seen; the reduction was 553 kJ (IDet+Diet) and 787 kJ (IDet). The rate (episodes/exposure year) of hypoglycaemic episodes was similar for the two groups: 25.47 episodes (IDet+Diet) and 23.30 episodes (IDet). No significant difference (*p* = 0.691) was seen between the groups; the estimated rate ratio (IDet/IDet+Diet) was 0.96 [0.79; 1.17].

**Conclusion:** An average weight reduction was observed regardless of dietary intervention when initiating insulin detemir in insulin-naïve type 2 diabetes subjects; the difference in weight change between the two groups was non-significant. The weight loss was most pronounced for subjects in the highest BMI strata. Glycaemic control as measured by HbA<sub>1c</sub> and FPG improved similarly in both groups.

**Figure 1: Mean observed change from baseline in body weight (kg) after 26 weeks by BMI strata.**



Clinical Trial Registration Number: NCT01232491

Supported by: Novo Nordisk

## 940

### Gradual intensification of premixed insulin lispro therapy vs basal ± mealtime insulin in patients with type 2 diabetes eating light breakfasts

D. Giugliano<sup>1</sup>, V. Woo<sup>2</sup>, S. Shah<sup>3</sup>, M. Tracz<sup>4</sup>, C. Mistodis<sup>5</sup>, A. Calle-Pascual<sup>6</sup>, R. Duarte<sup>7</sup>, R. Sari<sup>8</sup>, J. Deinhard<sup>9</sup>, A.O. Jiletcovici<sup>10</sup>, S. Wille<sup>10</sup>, J. Kiljanski<sup>10</sup>; <sup>1</sup>Second Univ. of Naples, Italy, University of Manitoba Health Sciences Center, Winnipeg, Canada, <sup>2</sup>Diabetes Action Centre, Mumbai, India, <sup>3</sup>Dept of Gastroenterology and Metabolic Diseases, Med University Warsaw, Poland, <sup>4</sup>Spitalul Clinic de Urgenta "Sf. Apostol Andrei", Galati, Romania, <sup>5</sup>Servicio Endocrinologia, Hospital Clinico San Carlos, Madrid, Spain, <sup>6</sup>Eli Lilly and Company, Lisboa, Portugal, <sup>7</sup>Faculty of Medicine, Akdeniz University, Antalya, Turkey, <sup>8</sup>Accovion GmbH, Eschborn, Germany, <sup>9</sup>Eli Lilly and Company, Vienna, Austria.

**Background and aims:** Insulin therapy in type 2 diabetes mellitus (T2DM) should be tailored to individual patients' (pts) needs. We tested if, in those eating light breakfasts, premixed insulin analogues may be an option to substitute basal ± mealtime insulin.

**Materials and methods:** This randomized, open-label, 48-week, parallel arm study compared 2 algorithms of initiation and then intensification of insulin therapy. One, 2 or 3 injections of insulin lispro mix 25 and/or insulin lispro mix 50 (lispro mix algorithm; LM) OR glargine ±1, 2 or 3 injections of insulin lispro (basal ± meal-time insulin algorithm; BM) were used in T2DM pts not controlled with oral antihyperglycaemic medications, consuming <15% daily calories at breakfast. The objective was to test the hypothesis that LM was non-inferior to BM for glycaemic control, measured by HbA1c after 48 weeks, assessed using ANOVA adjusted for baseline HbA1c and with a non-inferiority margin of 0.4%. Post-hoc analyses assessed the mean number of injections/day and percentage of pts achieving <7.0% HbA1c without hypoglycaemia.

**Results:** Pts (n=344: 176 [51%] females, mean [SD] age 54.3 [8.8] years, BMI 29.4 [4.6] kg/m<sup>2</sup>, baseline HbA1c 9.02 [0.97] %) were randomized to LM (n=171) or BM (n=173). In the per-protocol analysis (n=230) "LS means" (95%CI) endpoint HbA1c were 7.40 (7.15 - 7.65) and 7.55 (7.27 - 7.82) in LM and BM arms, respectively. The between-treatment difference was -0.14% (-0.42, 0.13); non-inferiority was met and confirmed in the full analysis set (n=321). Mean HbA1c changes at Week 48 were -1.68% (1.35) (LM) and -1.66% (1.31) (BM); p=0.967. Mean number of insulin injections/day at Week 48 was 1.96 in the LM and 1.99 in the glargine arm. HbA1c targets of <7.0% were achieved by 48.2% (LM) vs. 36.2% (BM) and ≤6.5% were achieved by 24.8% (LM) vs. 18.5% (BM) of pts. The percentage of pts achieving HbA1c <7.0% without hypoglycaemia was 13.1% and 8.5%, respectively at Week 48; p=0.251. SMBG profiles, body weight changes of +2.31 (3.3) kg and +2.32 (3.7) kg, and total insulin doses of 46.20 (28.4) IU/day and 46.45 (31.4) IU/day at Week 48 were similar in LM and BM, respectively. Overall (65% vs. 60%) and severe hypoglycaemia rate (2% vs. 4%) were similar in LM and BM groups (p=0.75); however, more LM pts had nocturnal symptomatic episodes (27.8% vs. 17.9%; p=0.039).

**Conclusion:** This study met the primary objective of establishing non-inferiority of an insulin mixtures-based regimen to a basal ± meal-time insulin regimen in achieving glycaemic control of T2DM patients eating a light breakfast, which is peculiar in countries with a Mediterranean diet.

Clinical Trial Registration Number: NCT00664534

Supported by: Eli Lilly and Company

## 941

### The way of insulin introduction more than the target glucose level determines the outcomes of critically ill patients with type 2 diabetes mellitus

N.G. Belyaeva, E.S. Komissarova, L.G. Strongin, S.A. Tezyaeva; State Medical Academy, Nizhny Novgorod, Russian Federation.

**Background and aims:** NICE-SUGAR has shown worsening of outcomes in maintenance of blood glucose level less than 6,1 mmol/l in critically ill patients with type 2 diabetes mellitus. The purpose of the present research is to compare the outcomes in different target glucose levels: 6,5-8,5 mmol/l and 8,6-11,0 mmol/l considering the way of insulin therapy.

**Materials and methods:** At the time of admission to the intensive care unit, 88 patients with various surgical pathology and type 2 diabetes mellitus, or hyperglycemia >11 mmol/l were randomly assigned to 4 groups. In group 1 (n=25) and 2 (n=20) the target glucose level 6,5-8,5 mmol/l was supported. In group 3 (n=21) and 4 (n=22) the level of glycemia 8,6-11,0 mmol/l was maintained. There were no differences in the baselin characteristics between the groups. The control of blood glucose level during the first 72 hours was carried out by intravenous infusion of short - acting insulin (in group 1 and 3), and by subcutaneous injections (in group 2 and 4). Risk scoring was performed using Acute Physiology and Chronic Health Evaluation (APACHE) score. Quantity of hypoglycemias and 90 - day survival rate were estimated.

**Results:** There were no significant differences between groups 1,2,3,4 in symptomatic hypoglycemias (p=0,5). Hypoglycemias less than 3,9 mmol/l were more often in the groups of subcutaneous injections (p=0,045). Parameters of gravity according to the APACHE score have decreased in group 1 on 10,0 (5,9-11,5) points, in group 3 - on 8,1 (5,9-9,6) points, in group 4 - on 6,9 (6,0-9,3), in group 2 - on 6,3 (5,2-11,3) points. 90-day survival rate shows the dependence only from the way of introduction of insulin 96%,50%,97,6%, 54,5% in groups 1,2,3,4 accordingly (p=0,03).

**Conclusion:** 90- day survival rate and dynamics of gravity according to APACHE score in critically ill patients with type 2 diabetes mellitus was significantly better, hypoglycemic effects were observed less often in the groups of insulin injections in both target glucose levels. However there were no significant differences in these parameters between groups with different target glucose levels when we used the same method of insulin treatment,

## 942

### Reduced physical activity one year after initiation of insulin therapy in patients with type 2 diabetes mellitus: results of a prospective study

H. Jansen, G. Vervoort, C. Tack; Radboud University Nijmegen Medical Centre, Netherlands.

**Background and aims:** Insulin therapy is frequently needed to achieve adequate glycaemic control in patients with type 2 diabetes mellitus (T2DM), but often at the expense of significant weight gain. Weight gain is partly explained by correction of glucose control, but other factors are largely unknown. We hypothesized that a decrease in physical activity may contribute to insulin-associated weight gain. This hypothesis was prospectively tested in a group of patients starting on insulin therapy.

**Materials and methods:** A total of 58 insulin-naïve patients with type 2 diabetes mellitus (M:F 32:26, mean age 61 ± 10 yr; mean diabetes duration 9 yr) were included. Physical activity was measured quantitatively using a SenseWear Pro Armband™ at baseline and after 12 months of insulin therapy. In addition, body weight, insulin dose and glycaemic control (HbA1c) were determined before and after 12 months of insulin therapy.

**Results:** After 12 months insulin therapy, mean body weight had increased by 3.6 ± 5.0 kg (SD) (range -5.8 to +21.0 kg; 55 % of the patients showed pronounced weight gain of ≥ 3 kg); HbA1c decreased from 8.9 ± 1.7 to 7.5 ± 1.1 % (both P < 0.05). Mean insulin dose at 12 months was 48 ± 33 U/day (0.51 U/kg insulin). Mean physical activity expressed as metabolic equivalent (METs) was significant lower at 12 months of insulin therapy compared to baseline (1.44 ± 0.29 vs. 1.36 ± 0.28; P < 0.05). Also the average number of steps per

day significantly diminished after 12 months of insulin treatment compared to baseline (baseline:  $6757 \pm 3432$  vs.  $5952 \pm 2755$  at 12 months of insulin,  $P < 0.05$ ). There was no correlation between the change in body weight over 12 months and the change in physical activity level.

**Conclusion:** Physical activity is decreased after 12 months of insulin therapy in patients with type 2 diabetes mellitus. Whether the decrease in physical activity is partly a cause of insulin-induced weight gain or merely a consequence remains to be determined.

*Clinical Trial Registration Number:* NCT00781495

## PS 077 Insulin therapy: clinical perspectives

### 943

**A pooled analysis of patients with type 2 diabetes mellitus to evaluate the efficacy and safety of a basal-bolus regimen across different age groups**

A. Cali<sup>1</sup>, G. Dailey<sup>2</sup>, J. Lin<sup>3</sup>, E. Wang<sup>4</sup>;

<sup>1</sup>Sanofi, Paris, France, <sup>2</sup>Scripps Clinic and Research Foundation, La Jolla, USA, <sup>3</sup>Novosys Health, Flemington, USA, <sup>4</sup>Sanofi, Bridgewater, USA.

**Background and aims:** Basal-bolus (mealtime bolus doses of insulin added to basal insulin) refers to a type of diabetes treatment intensification regimen that is prescribed for both young and older patients with type 2 diabetes mellitus. We evaluated the efficacy and safety of adding mealtime insulin glulisine to basal insulin across 3 different age groups ( $< 55$ ,  $55$ – $64$ , and  $\geq 65$  years).

**Materials and methods:** Data were pooled from 5 randomised, multicentre studies examining patients with poor glycaemic control who were initiated on basal insulin to which insulin glulisine was added at mealtime, with age group ranges approximating tertiles. Patients were treated for up to 6 months. Demographic information and efficacy and safety data were collected at baseline and at each study's endpoint.

**Results:** A total of 1413 patients were analysed (49.5% female). A total of 32.2% ( $n = 455$ ) were  $< 55$  years of age, 42.6% ( $n = 602$ ) were  $55$  to  $64$  years of age, and 25.2% ( $n = 356$ ) were  $\geq 65$  years of age. Mean body mass index for those  $< 55$ ,  $55$  to  $64$ , and  $\geq 65$  years of age was  $35.6 \text{ kg/m}^2$ ,  $34.2 \text{ kg/m}^2$ , and  $32.0 \text{ kg/m}^2$ , respectively. The basal-bolus regimen resulted in significant decreases in  $\text{HbA}_{1c}$  between baseline and study end in all 3 age groups (all  $P < 0.001$ ; Table). Among the 3 age groups, this change was similar (Table). Target  $\text{HbA}_{1c}$  ( $< 7\%$ ) was reached by 45.5%, 50.3%, and 54.5% in the  $< 55$ ,  $55$  to  $64$ , and  $\geq 65$  year cohorts, respectively. Incidence of severe hypoglycaemia (as defined in each trial protocol) was similar (Table). There was weight gain in each age group ( $P < 0.001$ ), with significantly less weight gain in the two older groups.

**Conclusion:** This pooled analysis suggests that basal-bolus treatment with mealtime insulin glulisine added to basal insulin significantly and clinically reduces  $\text{HbA}_{1c}$  across different age groups, including the older groups, with similar rates of severe hypoglycaemia. In addition, there was less weight gain in the older patients compared to younger patients.

Age range, year	<55	55 to 64	$\geq 65$	P value (between age groups)
Patients, n	455	602	356	
$\text{HbA}_{1c}$ at baseline, %	8.1 (1.0)	7.8 (0.9)	7.7 (0.9)	$< 0.01$
$\text{HbA}_{1c}$ at endpoint, %	7.3 (1.1)	7.0 (0.9)	6.9 (0.8)	$< 0.01$
$\Delta \text{HbA}_{1c}$ between baseline and endpoint, %	-0.8* (1.1)	-0.8* (1.0)	-0.8* (1.0)	0.8
$\Delta$ Weight, kg	3.5 (4.8)	2.3 (4.4)	2.1 (3.8)	$< 0.01$
Severe hypoglycaemia, Events/Year	0.2 (1.0)	0.3 (2.3)	0.1 (0.6)	0.3

\*  $P < 0.001$ . All values are mean (SD), unless noted.

Supported by: Sanofi

### 944

**Relative contribution of basal and postprandial blood glucose to hyperglycaemia in patients (Pts) with type 2 diabetes mellitus enrolled in the START study**

S. Harris<sup>1</sup>, J.-F. Yale<sup>2</sup>, B. Abbaszadeh<sup>3</sup>, J. Stewart<sup>3</sup>, L. Berard<sup>4</sup>;

<sup>1</sup>Western University, London, <sup>2</sup>McGill University, Montreal, <sup>3</sup>Sanofi, Laval, <sup>4</sup>Health Science Centre, Winnipeg, Canada.

**Background and aims:** Recent studies have described the relative contribution of Basal Blood Glucose (BBG) and Postprandial Blood Glucose (PPBG) across various  $\text{HbA}_{1c}$  (A1c) values, but very few studies demonstrated the PPBG contribution in both insulin naïve (IN) and insulin treated (IT) Pts with T2DM. The START study compared a patient-driven algorithm for titrating insulin glargine (iGla) and insulin glulisine (iGlu) to a physician-driven algorithm for insulin intensification in Pts with T2DM not at target. The run-in phase (RI-P) of START study provided an opportunity to describe the relative



contributions of PPBG and BBG in IN and IT Pts utilizing 7-point glucose profiles prior to and following the initiation and/or optimization of iGla for 12 weeks.

**Materials and methods:** In this open-label, multicenter, randomized, comparative, Canadian study, uncontrolled Pts with T2DM receiving basal insulin  $\pm$  oral antihyperglycemic drugs (OADs) or IN on 2–3 OADs were eligible to participate. Pts entered RI-P, during which they received iGla using simple titration algorithm. At the end of RI-P, all Pts were on a stable dose of basal insulin. Patients with an A1c still above 7.0% were randomized into a patient or physician-driven algorithm using iGlu titration for 24 weeks to achieve optimal glycemic control. Pts with complete 7-point glucose profiles at baseline and at the end of the RI-P were included in this analysis. The area under the curve (AUC) was partitioned into three parts: area above the fasting plasma glucose (AUCp), area between the fasting plasma glucose and 5.5 mmol/L (AUCb), and the area below 5.5 mmol/L. The percent for the PPBG and BBG contribution were calculated as  $100 \times \text{AUCp}/(\text{AUCb} + \text{AUCp})$  and  $100 \times \text{AUCb}/(\text{AUCb} + \text{AUCp})$  respectively.

**Results:** There were 214 IN and 212 IT Pts whose mean ages (years) were 58.5 and 60.2 and percent males were 64.5 and 56.1, respectively. Mean A1c at baseline was 9.3 and 8.6 for IN and IT Pts respectively. After 12 weeks of initiating or optimizing iGla mean A1c and FPG change were -0.83% and -3.2 mmol/L. A1c reduction was higher in IN than IT (-1.21 vs -0.45). The result for PPBG and BBG contribution to hyperglycemia are shown in the table.

**Conclusion:** The RI-P results of the START study showed that the glucose profile of IN and IT Pts are different depending on their relative A1c levels. In IN Pts with A1c below 8.5%, the contribution of PPBG to hyperglycemia is higher than for A1c above 8.5%. In IT Pts, PPBG contribution to hyperglycemia is higher or equal to BBG contribution in Pts with A1c below 9%. Initiating or optimizing iGla as expected corrects for BBG hyperglycemia and increased PPBG contribution across all A1c levels. These findings suggest that depending on the A1c and whether or not a patient is already on basal insulin, an individualized approach should be considered for initiating or intensifying treatment in Pts with T2DM above the A1c of 7%.

	Baseline			Run-in phase Results 12 weeks following initiation or optimization of insulin glargine		
	Mean (SD) % of PPBG Contribution to hyperglycemia*			Mean (SD) % of PPBG Contribution to hyperglycemia*		
	Insulin Naive	Insulin treated	Total	Insulin Naive	Insulin treated	Total
HbA1c < 7%	--	--	--	81.0 (28.4)	85.1 (24.1)	82.8 (26.4)
HbA1c < 8%	28.9 (25.7)	60.5 (33.6)	51.8 (34.5)	69.0 (34.8)	75.9 (28.4)	72.4 (32.0)
8% $\geq$ HbA1c < 8.5%	36.8 (28.7)	51.1 (32.5)	43.8 (31.2)	75.0 (24.1)	74.0 (30.4)	74.4 (27.6)
8.5% $\geq$ HbA1c < 9%	27.7 (28.4)	49.6 (29.0)	38.5 (30.5)	68.9 (30.1)	69.3 (35.4)	69.1 (32.4)
9% $\geq$ HbA1c < 9.5%	23.8 (19.5)	46.2 (28.4)	34.3 (28.4)	76.8 (26.1)	65.1 (30.6)	71.3 (28.5)
HbA1c $\geq$ 9.5%	21.8 (19.0)	40.8 (30.5)	28.2 (25.0)	59.3 (28.6)	58.7 (32.5)	59.0 (30.5)

\* % of BBG Contribution to hyperglycemia is 100 - % PPBG

Clinical Trial Registration Number: NCT01013571

Supported by: Sanofi Canada

## 945

### Lower incidence of diabetic complications with insulin glargine versus basal human insulins in patients with type 1 or type 2 diabetes: a population-based cohort study in Italy

S. Cammarota<sup>1</sup>, D. Bruzzese<sup>2</sup>, A.L. Catapano<sup>3</sup>, A. Citarella<sup>1</sup>, L. De Luca<sup>1</sup>, L. Manzoli<sup>4</sup>, M. Masulli<sup>5</sup>, E. Menditto<sup>1</sup>, A. Mezzetti<sup>6</sup>, S. Riegler<sup>1</sup>, D. Putignano<sup>1</sup>, E. Tragni<sup>3</sup>, E. Novellino<sup>1</sup>, G. Riccardi<sup>5</sup>

<sup>1</sup>Center of Pharmacoeconomics and Drug Utilization, "Federico II" University, Naples, <sup>2</sup>Department of Preventive Medical Sciences, "Federico II" University, Naples, <sup>3</sup>Department of Pharmacological Sciences, University of Milan, <sup>4</sup>Section of Hygiene, Epidemiology, Pharmacology and Legal Medicine, G. D'Annunzio University Foundation, Chieti, <sup>5</sup>Department of Clinical and Experimental Medicine, "Federico II" University, Naples, <sup>6</sup>Clinical Research Centre, G. D'Annunzio, Chieti, Italy.

**Background and aims:** Several studies have demonstrated that the introduction of both rapid- and long-acting insulin analogues may help to achieve better glycemic control with less hypoglycemic episodes than the traditional human insulin (HI) formulations. It remains unknown whether insulin analogues are also better in reducing long-term diabetic complications. Aim of this study was to compare the use of insulin glargine and intermediate/long-acting HI in relation to the first occurrence of diabetes-related complications in a cohort of diabetic patients.

**Materials and methods:** A population-based cohort study was conducted using administrative data from four local health authorities in the Abruzzo Region (900,000 inhabitants). A record linkage-analysis was done to identify diabetic patients free of macrovascular disease at baseline and consistently treated with insulin glargine or intermediate/long-acting HI during a 3-year follow-up. Patients were followed from January 1, 2006 until the date of hospital admission for any diabetes-related complications, censoring (death or emigration), or December 31, 2008, whichever occurred first. HRs and 95% CIs of any diabetic complication and macrovascular, microvascular and metabolic complications were estimated separately using Cox proportional hazard models adjusted for age, gender, previous microvascular or metabolic complications, concomitant drugs and anti-diabetic drugs use. Propensity score matching was also used to adjust for differences in cardiovascular risk markers between the two treatment groups.

**Results:** Overall, 1,921 diabetic patients were included: 744 intermediate/long-acting HI users and 1,177 insulin glargine users. During the 3-year follow-up, 209 incident events of any diabetes-related complication in the intermediate/long-acting HI group (28.1%, 123 events per 1,000 person-years at risk) and 159 in the insulin glargine group (13.5%, 50 events per 1,000 person-years at risk) occurred. After adjustment for covariates, insulin glargine users had a HR (95% CI) of 0.58 (0.45–0.74) for any diabetes-related complication and HRs of 0.63 (0.47–0.86), 0.55 (0.32–0.96) and 0.38 (0.19–0.73) for macrovascular, microvascular and metabolic complications, respectively, as compared with intermediate/long-acting HI users. Propensity score-based matched-pair analyses supported these findings.

**Conclusion:** Our results suggest that the use of insulin glargine compared with traditional basal insulins is associated with a lower risk of diabetic complications; long-term controlled intervention trials are needed to confirm this hypothesis.

Supported by: "Federico II" University of Naples

## 946

### Insulin glargine metabolite M1 is the principal insulin component circulating in plasma of young children with type 1 diabetes: Results from the PRESCHOOL study

T. Danne<sup>1</sup>, P. Johnston<sup>2</sup>, G. Xiang<sup>3</sup>, A. Cali<sup>4</sup>, M.-P. Dain<sup>4</sup>, A. Philotheou<sup>5</sup>, <sup>1</sup>Kinder- und Jugendkrankenhaus 'Auf der Bult', Hannover, Germany, <sup>2</sup>Sanofi, Bridgewater, USA, <sup>3</sup>Fewmo Technology Co.LTD, Beijing, China, <sup>4</sup>Sanofi, Paris, France, <sup>5</sup>UCT Private Academic Hospital, Cape Town, South Africa.

**Background and aims:** The principal circulating component in plasma of adults is insulin glargine metabolite M1, but the metabolism of insulin glargine has not been studied in young children. The objective of this study was to examine the metabolism of insulin glargine in young children in order to rule out accumulation of insulin glargine in this population. Ct.gov NCT00993473.

**Materials and methods:** Children with type 1 diabetes from the PRESCHOOL study, aged 1 to 6 years, who were treated with insulin glargine for 24 weeks (n = 61) had blood samples drawn at Weeks 1, 2, and 4 approximately 24 hours after the last dose for pharmacokinetic analysis of plasma levels of glargine and its metabolites. C<sub>trough</sub> values for insulin glargine and its metabolites M1 and M2 were determined using LC-MS/MS. The lower limit of quantification was 0.2 ng/mL for parent and each metabolite.

**Results:** Insulin glargine metabolite M1 was the principal circulating component in plasma. Mean (SD) plasma M1 C<sub>trough</sub> values were 0.580 (0.786), 0.458 (0.700), and 0.452 (0.583) ng/mL at Weeks 1, 2, and 4, respectively, indicating no accumulation of M1. The standard deviation was observed to not change over time. Mean insulin glargine parent and metabolite M2 concentrations were below the level of quantification. Similar results have been observed in adults.

**Conclusion:** For young children with type 1 diabetes, the principal circulating insulin component in plasma was insulin glargine metabolite M1, known to be the pharmacologically active component. Since M1 does not differ from human insulin in terms of binding to IGF-1R and in mitogenesis, these data provide additional evidence for the safety profile of insulin glargine in young children.

Clinical Trial Registration Number: NCT00993473

Supported by: Sanofi

## 947

**Improved glycaemic control with once-daily insulin glargine in people with type 2 diabetes inadequately controlled on insulin detemir/OAD combination therapy (RESOLUTE)**L. Lieve<sup>1</sup>, M. Rodriguez<sup>2</sup>, L. Czupryniak<sup>3</sup>, W. Landgraf<sup>4</sup>, V. Pilorget<sup>5</sup>, M.-P. Dain<sup>5</sup>, M. Kvapil<sup>6</sup><sup>1</sup>Maxima Medical Centre, Eindhoven, Netherlands, <sup>2</sup>University Hospital, Mendoza, Argentina, <sup>3</sup>University of Lodz, Poland, <sup>4</sup>Sanofi, Frankfurt, Germany, <sup>5</sup>Sanofi, Paris, France, <sup>6</sup>University Hospital Motol, Prague, Czech Republic.

**Background and aims:** Evidence from a randomised clinical trial indicates clinically relevant differences in efficacy between basal insulin glargine (GLA) and insulin detemir (DET) when used once-daily. We investigated whether patients with type 2 diabetes (T2D) inadequately controlled with DET therapy in combination with oral anti-diabetic agents (OADs) would benefit from switching to GLA treatment in everyday clinical practice.

**Materials and methods:** RESOLUTE was a multi-national (5 countries), non-interventional, 6-month, prospective, observational study performed between June 2010 and November 2011 in 88 office- and hospital-based diabetes centres. Patients with T2D treated for  $\geq 3$  months with DET (QD or BID) combined with OADs and having an HbA<sub>1c</sub>  $> 7\%$  to  $10\%$  were eligible when switched to GLA at the physician's discretion. The primary endpoint was the change in HbA<sub>1c</sub> after 6 months. The main secondary endpoints included fasting plasma glucose (FPG), insulin dose, body weight, hypoglycaemia over the last month and adverse events.

**Results:** Of 564 patients included in the study, 511 were eligible for analysis, of whom 59% previously received QD DET and 40% BID DET in addition to OADs (92% received metformin and/or SU). Patient characteristics (61% female) were (mean [SD]): age, 61.9 (10.1) years; BMI, 30.9 (5.1) kg/m<sup>2</sup>; duration of diabetes, 9.2 (5.9) years. Patients were on DET therapy for 14.4 (12.7) months with 28.4 (17.1) U/day. GLA was initiated once-daily at bedtime (79%), dinner (11%) or breakfast (8%) in 98% of patients. After a mean follow-up period of 186 (17) days, mean HbA<sub>1c</sub> decreased from 8.37% (0.77) to 7.32% (0.95) ( $P < 0.0001$ ) and mean FPG from 8.9 (2.1) mmol/L to 6.8 (1.7) mmol/L ( $P < 0.0001$ ). Glycaemic improvements were more pronounced in former BID DET patients (table). At study end, 40% of patients reached HbA<sub>1c</sub>  $< 7\%$  and 25% were between 7% and 7.5%. OAD co-medication was continued in a comparable manner during follow-up. Mean body weight slightly decreased (-0.2 [2.5] kg), and the percentage of patients with reported symptomatic (9.4%), nocturnal (3.1%) and severe (0%) hypoglycaemia in the last month of GLA therapy was consistently lower compared with the last month of previous DET treatment (16.4%, 8.2% and 1.2%, respectively). The number of patients who reported adverse events was low with GLA (2.4%).

**Conclusion:** Under real-life conditions in poorly controlled patients with T2D, replacement of QD or BID DET with once-daily GLA resulted in a clinically relevant improvement in glycaemic control without increased hypoglycaemia risk or weight gain.

Parameter	GLA QD, switched from:		
	Overall DET (n = 511)	QD DET (n = 302)	$\geq$ BID DET (n = 209)
HbA <sub>1c</sub> (%)			
Baseline	8.37 (0.77)	8.35 (0.80)	8.39 (0.72)
6 months	7.32 (0.95)	7.50 (0.96)	7.06 (0.87)
Change	-1.05 (0.88)*	-0.85 (0.86)*	-1.33 (0.84)*
FPG (mmol/L)			
Baseline	8.9 (2.1)	8.9 (2.3)	8.9 (1.7)
6 months	6.8 (1.7)	7.1 (2.0)	6.4 (1.2)
Change	-2.1 (2.2)*	-1.7 (2.4)*	-2.5 (1.9)*
DET last daily dose (U)	28.4 (17.1)	19.5 (10.0)	41.3 (16.9)
GLA daily dose (U)			
Baseline	24.3 (12.9)	19.6 (9.6)	31.2 (14.0)
6 months	30.3 (15.5)	23.5 (10.9)	40.1 (16.0)
Change vs DET	+1.8 (8.4)*	+3.9 (6.7)*	-1.3 (9.5)**
<u>Overall symptomatic hypoglycaemia:- last month of DET (%)</u>			
- last month of GLA (%)	.	.	.
<u>Symptomatic.</u>			
- nocturnal	16.4	14.2	19.6
- hypoglycaemia:	9.4	7.3	12.4
- last month of DET (%)	.	.	.
- last month of GLA (%)	8.2	7.3	9.6
- last month of GLA (%)	3.1	2.3	4.3
- last month of GLA (%)	.	.	.
- Severe	1.2	1.3	1.0
- hypoglycaemia:	0	0	0
- last month of DET (%)	.	.	.
- last month of GLA (%)	.	.	.
- last month of GLA (%)	.	.	.

Values are mean (SD). \* $P < 0.0001$ ; \*\* $P = 0.13$ , baseline vs 6 months (Wilcoxon signed rank test)

Supported by: Sanofi

## 948

**Study of Once-Daily Levemir (SOLVE™): Patient quality of life and physician resource utilisation of once-daily insulin detemir in Chinese patients with type 2 diabetes**C. Pan<sup>1</sup>, J. Lu<sup>1</sup>, P. Wang<sup>2</sup>, Q. Ji<sup>3</sup>, L. Ji<sup>4</sup><sup>1</sup>Chinese PLA General Hospital, Beijing, <sup>2</sup>Tianjin Medical University Metabolic Diseases Hospital, Tianjin, <sup>3</sup>Xijing Hospital affiliated to 4th Military Medical University, Xi'an, <sup>4</sup>Peking University People's Hospital, Beijing, China.

**Background and aims:** Barriers to insulin initiation in people with type 2 diabetes mellitus (T2DM) include fear of increased treatment complexity by both patients and healthcare professionals, and a perceived lack of time and resources by primary care physicians. SOLVE™ was a 24-week international observational study of insulin detemir initiation in patients with T2DM previously treated with one or more oral antidiabetic drugs (OADs) across 10 countries. This abstract reports the effect of insulin detemir initiation on patient quality of life and resource utilization based on a sub-analysis of data from China.

**Materials and methods:** Patient quality of life was assessed at baseline and end of study using the Insulin Treatment Appraisal Scale (ITAS) - a self-assessment questionnaire comprising 20 items (16 negative items and 4 positive items) with 5 categories of 'strongly disagree', 'disagree', 'neither agree nor disagree', 'agree' and 'strongly agree'. Physician resource utilization was self-assessed by a questionnaire.

**Results:** A total of 3272 patients were enrolled in China. Of these, 2988 patients (91.3%) completed the 24-week study (58% male, age 56.2 $\pm$ 10.8 years, BMI 25.3 $\pm$ 3.3 kg/m<sup>2</sup>, duration of diabetes 7.1 $\pm$ 5.2 years, OAD therapy 6.3 $\pm$ 5.1 years). During the study period, neither serious adverse drug reactions nor major hypoglycemic events were reported. Insulin dose at the end

of the study was  $0.24 \pm 0.11$  U/kg.  $HbA_{1c}$  improved by  $-1.2 \pm 1.7\%$  ( $p < 0.001$ ). The ITAS questionnaire was completed by 2872 patients. The main concerns of initiating insulin at baseline were (reported as percentages of patients agree or strongly agree): item 18: 'Being on insulin causes family and friends to be more concerned about me' (64.7%), item 1: 'Taking insulin means I have failed to manage my diabetes with diet and tablets' (53.3%) and item 20: 'Taking insulin makes me more dependent on my doctor' (52.3%). After the 24-week treatment, the largest changes in attitudes occurred in relation to the following items: Item 19: 'Taking insulin means help to improve my energy level' (from 59.0% at baseline to 77.5% at Week 24); Item 6: 'I am afraid of injecting myself with a needle' (from 34.5% to 17.4%); and Item 1: 'Taking insulin means I have failed to manage my diabetes with diet and tablets' (from 53.3% to 44.8%); all of which improved after initiating insulin. A total of 3272 physician resource utilisation questionnaires were collected. It was reported that NovoPen 4 was the preferred device (45.0%). The time taken to teach patients to self-inject and perform dose self-adjustment was  $13.4 \pm 9.79$  minutes and  $11.4 \pm 9.32$  minutes, respectively. Only 2.2% of physicians reported the use of insulin detemir by patients as difficult or very difficult; and only 8.8% of physicians reported being dissatisfied or very dissatisfied with  $HbA_{1c}$  target achieved by the end of the study.

**Conclusion:** Initiating insulin detemir therapy once daily as add-on to OADs was safe and effective for Chinese patients with T2DM, and it was not perceived as being difficult by patients as reported by physicians. Patients' perceptions and satisfaction of insulin treatment improved after initiation of insulin.

*Clinical Trial Registration Number:* NCT00825643

*Supported by:* Novo Nordisk

## 949

### Superior glycaemic control with once-daily IDegAsp vs insulin glargine in Japanese adults with type 2 diabetes mellitus inadequately controlled with OADs: a randomised controlled, phase 3 trial

S. Nakamura<sup>1</sup>, Y. Ono<sup>2</sup>, R. Rabol<sup>3</sup>, L. Endahl<sup>3</sup>, Y. Onishi<sup>4</sup>

<sup>1</sup>Internal Medicine, Iryo Houjin Shadan Kowakai Heiwadai Hospital, Miyazaki, Japan, <sup>2</sup>Takagi Hospital, Fukuoka, Japan, <sup>3</sup>Novo Nordisk A/S, Søborg, Denmark, <sup>4</sup>Division of Diabetes and Metabolism, Institute for Adult Diseases, Asahi Life Foundation in Tokyo, Japan.

**Background and aims:** Insulin degludec (IDeg) is a new basal insulin that forms soluble multi-hexamers upon s.c. injection, resulting in an ultra-long and consistent glucose-lowering effect. Insulin degludec/insulin aspart (IDegAsp) is a soluble co-formulation of IDeg (70%) and insulin aspart (30%) that provides both mealtime and basal insulin coverage. We investigated the efficacy and safety of IDegAsp in insulin-naïve Japanese adults with type 2 diabetes inadequately controlled on oral antidiabetic drugs (OADs) in a phase 3, 26-week, open-label, treat-to-target trial.

**Materials and methods:** Participants (mean: 60.5 years old;  $HbA_{1c}$  8.4%; fasting plasma glucose [FPG] 9.0 mmol/L; BMI 25.1 kg/m<sup>2</sup>; duration of diabetes 11.7 years) were randomised to once-daily injections of IDegAsp ( $n=147$ ) or insulin glargine (IGlar;  $n=149$ ), both with/without up to 2 OADs (excluding sulphonylureas, dipeptidyl peptidase-4 inhibitors and glinides). IDegAsp was injected before the largest meal of the day at the discretion of each participant (and maintained throughout the trial); IGlar was injected at the same time each day according to label. Both insulins were titrated to an FPG  $< 5$  mmol/L. **Results:** After 26 weeks, mean  $HbA_{1c}$  was 7.0% with IDegAsp and 7.3% with IGlar. Analysis of change in  $HbA_{1c}$  from baseline to end-of-trial demonstrated that IDegAsp was superior to IGlar (estimated treatment difference (ETD) IDegAsp-IGlar:  $-0.28\%$  [-0.46; -0.10]<sub>95% CI</sub>,  $p < 0.001$ ). At end-of-trial, a greater proportion of subjects achieved  $HbA_{1c} < 7.0\%$  with IDegAsp (58.5%) vs. IGlar (40.3%). Mean FPG was similar for IDegAsp and IGlar (5.7 vs. 5.6 mmol/L; ETD IDegAsp-IGlar: 0.15 mmol/L [-0.29; 0.60],  $p=NS$ ). No severe hypoglycaemia was reported. Confirmed hypoglycaemia (PG  $< 3.1$  mmol/L) was reported for 44% of subjects in both groups; IDegAsp was associated with a numerically lower (27%) rate of confirmed hypoglycaemia than IGlar (estimated rate ratio (ERR) IDegAsp/IGlar: 0.73 [0.50; 1.08]  $p=NS$ ). A significantly greater proportion of subjects achieved  $HbA_{1c} < 7.0\%$  at end-of-trial (without confirmed hypoglycaemia in the last 12 weeks of treatment) with IDegAsp compared to IGlar (43% vs. 25%; Odds Ratio 2.21 [1.25; 3.92],  $p=0.003$ ). The rate of nocturnal confirmed hypoglycaemia (confirmed hypoglycaemia with onset between 00:01-05:59 h) was numerically lower (by 25%) with IDegAsp than IGlar (RR: 0.75 [0.34; 1.64],  $p=NS$ ). Mean daily insulin doses were similar between groups at the end-of-trial (IDegAsp: 28 U; IGlar: 29 U) as were increases in body weight from baseline (0.7 kg for both

groups). Overall rates of adverse events were similar between groups with no treatment-specific pattern or clustering.

**Conclusion:** IDegAsp dosed once daily with the largest meal of the day provided superior long-term glycaemic control with similar FPG to IGlar at a numerically lower rate of overall and nocturnal hypoglycaemia.

*Clinical Trial Registration Number:* NCT01272193

*Supported by:* Novo Nordisk

## 950

### Comparing the efficacy of biphasic insulin aspart-30 and insulin detemir given in the morning as an add-on to type 2 diabetic patients treated with OHA by using CGM

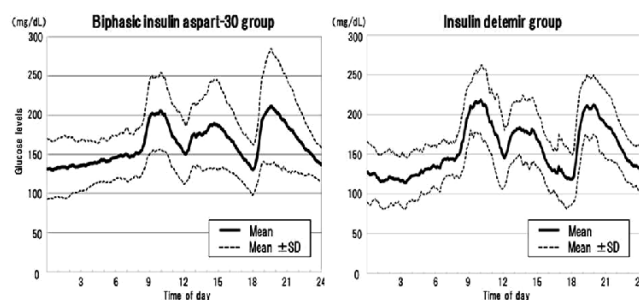
D. Tsujino, R. Nishimura, C. Seo, K. Andou, A. Morimoto, K. Utsunomiya; Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan.

**Aims:** To compare the efficacy of biphasic insulin aspart-30 (A) and insulin detemir (D) given in the morning as an add-on to type 2 diabetic patients treated with oral hypoglycaemic agents (OHA).

**Methods:** This study enrolled 25 poorly controlled type 2 diabetic patients ( $8\% < HbA_{1c} < 11\%$ ) being treated with OHA mono- or combination therapy consisting of sulphonylureas, thiazolidinediones or biguanides. The patients were randomly assigned to A or D, which were administered in the morning as an add-on to OHA mono- or combination therapy, with the baseline-dosing methods maintained after allocation. After adjusting their insulin dosages over a period of  $\geq 2$  months to achieve predinner glucose levels  $< 130$  mg/dL, the patients were hospitalised and monitored by CGM for 3 days. Day 2 CGM data were subjected to analysis for comparison. The t-test was used to compare the 24-hour mean glucose levels and their standard deviations (SDs) in groups A and D; the mean amplitude of glucose excursions (MAGE), the proportion of time in hypoglycaemia (180 mg/dL), the postprandial peak glucose levels, the range of postprandial glucose increases and the time from preprandial glucose levels to postprandial peak glucose levels. The present study was conducted with the approval of the Institutional Review Board at our university.

**Results:** There was no significant difference in the patients' backgrounds between groups A and D [males/females, 7/5 vs. 9/4; age (mean  $\pm$  SD),  $59.9 \pm 10.2$  vs.  $61.3 \pm 6.9$  years; and BMI,  $26.9 \pm 4.2$  vs.  $24.8 \pm 3.5$  kg/m<sup>2</sup>], as well as in baseline  $HbA_{1c}$  levels ( $9.16 \pm 0.49$  vs.  $9.02 \pm 0.42\%$ ), insulin dosages at admission ( $0.117 \pm 0.067$  vs.  $0.115 \pm 0.071$  U/kg per day) or decreases in  $HbA_{1c}$  from the start of insulin to admission ( $-1.17 \pm 0.43$  vs.  $-1.14 \pm 0.75\%$ ). The 24-hour mean glucose levels and their SDs were not significantly different between groups A and D ( $162 \pm 29$  vs.  $155 \pm 16$  mg/dL and  $33.8 \pm 16.1$  vs.  $42.1 \pm 9.1$  vs., respectively). The MAGE was significantly lower in group A ( $75 \pm 35$ ) than in group D ( $103 \pm 13$ ) ( $P = 0.020$ ), and the time in hypoglycaemia tended to be shorter in group A ( $0.4 \pm 1.4$  min) than in group D ( $16.5 \pm 27.3$ ) ( $P = 0.055$ ), whereas the time in hyperglycaemia was not significantly different. Postprandial glucose increases were significantly narrower in range after breakfast in group A ( $63 \pm 34$ ) than in group D ( $105 \pm 34$ ) ( $P = 0.005$ ), with the postprandial peak glucose levels after breakfast tending to be lower in group A ( $210 \pm 49$  mg/dL) than in group D ( $243 \pm 33$  mg/dL) ( $P = 0.055$ ). The time to postprandial peak glucose levels tended to be shorter in group A than in group D (breakfast,  $86 \pm 25$  min vs.  $110 \pm 36$ ,  $P = 0.066$ ; dinner,  $74 \pm 34$  min vs.  $101 \pm 24$ ,  $P = 0.033$ ).

**Conclusion:** A comparison of glycaemic variations in type 2 diabetic patients by CGM demonstrated significantly lower MAGE with A than with D; the difference was mainly due to significantly greater reductions in glucose increases after breakfast.



*Clinical Trial Registration Number:* UMIN000004396

*Supported by:* Japan Diabetes Foundation



## 951

**Predictors of good blood glucose control in people receiving biphasic insulin aspart 30 premix insulin: data from the A<sub>1</sub>chieve study**P.D. Home<sup>1</sup>, Z.A. Latif<sup>2</sup>, P. Soewondo<sup>3</sup>, G. Gonzalez-Galvez<sup>4</sup>, V. Prusty<sup>5</sup>, Y. Li<sup>6</sup>;<sup>1</sup>Institute of Cellular Medicine – Diabetes, Newcastle University, UK,<sup>2</sup>BIRDEM, Dhaka, Bangladesh, <sup>3</sup>Faculty of Medicine, Jakarta, Indonesia,<sup>4</sup>Instituto Jalisciense de Investigacion en Diabetes y Obesidad, SC, Jalisco, Mexico, <sup>5</sup>Novo Nordisk Pharmaceuticals, Zurich, Switzerland, <sup>6</sup>Peking Union Medical College Hospital, Beijing, China.

**Background and aims:** Premix insulins, including biphasic insulin aspart 30, are widely used to enable the effective management of both basal and prandial glucose. Target HbA<sub>1c</sub> levels have been shown to be achievable in up to 66% of people with type 2 diabetes. The present subgroup analysis of the A<sub>1</sub>chieve study investigated which factors predicted the achievement of an HbA<sub>1c</sub> level of <7.0 % in a large population starting aspart premix 30.

**Materials and methods:** A<sub>1</sub>chieve was an international, prospective, multi-centre, non-interventional, 24-week study evaluating the safety and clinical effectiveness of insulin analogues in 66 726 people with type 2 diabetes. In this subanalysis, data from people receiving aspart premix 30 were examined to elucidate baseline characteristics which were statistically correlated with attainment of good blood glucose control, defined as HbA<sub>1c</sub> <7.0 %, at week 24. All statistical analyses were two sided, using a pre-specified 5% significance level, unless otherwise stated.

**Results:** Data from 29 834 participants begun on aspart premix 30 were analysed. Of these 9387 people achieved HbA<sub>1c</sub> <7.0 % by week 24. In this group HbA<sub>1c</sub> fell from 9.0 (SD 1.8) to 6.4 (0.4) % (mean change -2.6 [1.8] %), and fasting plasma glucose (FPG) from 10.4 (3.2) to 6.3 (1.1) mmol/l (change -4.1 [3.2] mmol/l). Multivariate analysis indicated that, after adjustment for differences between regions, the factors identified as predictors of an HbA<sub>1c</sub> of <7.0 % were shorter duration of diabetes, lower baseline BMI, HbA<sub>1c</sub> and triglycerides, and a lower prevalence of microvascular complications (Table). Age, FPG, postprandial glucose and systolic blood pressure at baseline did not emerge as independent predictors.

**Conclusion:** Several factors did predict achievement of target HbA<sub>1c</sub> levels in people with type 2 diabetes starting aspart premix 30. These seem to be markers of lesser overall metabolic disturbance and shorter duration of diabetes.

	Odds ratio (95% CI)	p
Duration of diabetes (per yr)	0.98 (0.97;0.99)	<0.001
BMI (per kg/m <sup>2</sup> )	0.98 (0.97;0.99)	0.01
HbA <sub>1c</sub> (per % unit)	0.77 (0.75;0.80)	<0.001
Quality of life (per 10% EQ-5D)	1.04 (1.002;1.09)	0.04
Total serum cholesterol (per mmol/l)	1.07 (1.02;1.12)	0.01
Serum triglycerides (per mmol/l)	0.90 (0.85;0.95)	<0.001
Microvascular complications (yes/no)	0.93 (0.88;0.99)	0.02
Baseline predictors of HbA <sub>1c</sub> <7.0 % 24 weeks after starting aspart premix 30		

Clinical Trial Registration Number: NCT00869908

Supported by: Novo Nordisk

## PS 078 Psychological aspects I

## 952

**Association of glycaemic control and hypoglycaemia with personality traits and resilience of patients with diabetes**W.C. Kewelow<sup>1</sup>, F. Zillich<sup>2</sup>, K. Wick<sup>2</sup>, N. Müller<sup>1</sup>, C. Kloos<sup>1</sup>, T. Lehmann<sup>3</sup>, G. Wolf<sup>1</sup>, U.A. Müller<sup>1</sup>;<sup>1</sup>Endocrinology and Metabolic Diseases, Internal Medicine III, University Hospital, <sup>2</sup>Institute of Psychosocial Medicine and Psychotherapy, University Hospital, <sup>3</sup>Institute of Medical Statistics, Information Sciences and Documentation, University Hospital, Jena, Germany.

**Background and aims:** Structured therapy and education programs for patients with type 1 (T1DM) and type 2 diabetes (T2DM) have been shown to improve HbA<sub>1c</sub> and to reduce rates of hypoglycaemia as well as the individual dose adjustment. But not all patients succeed to reach their treatment goals after participating in such a program. Personality traits have been shown to be a predictor for long-term mortality and complications in patients with chronic diseases. We assessed the personality traits neuroticism (NE), extraversion (EX), conscientiousness (CO), agreeableness (AG) and openness (OP) as well as resilience (RES) to explain therapy success or failure.

**Materials and methods:** We conducted a cross-sectional study of patients with T1DM and T2DM in a tertiary care centre in Germany during a period of three months. We assessed the short version of the Big Five Inventory (BFI-S), resilience scale (RS-13), treatment satisfaction (DTSQ standard) and social status in 669 patients (162 T1DM, age 52.17, duration of diabetes 20.05 years, HbA<sub>1c</sub> 7.51%, BMI 26.6 kg/m<sup>2</sup>, 507 T2DM, age 67.43, duration of diabetes 15.4 years, HbA<sub>1c</sub> 7.31%, BMI 32.9 kg/m<sup>2</sup>). Quality of diabetes care was measured by HbA<sub>1c</sub>, blood pressure, BMI and rates of serious and severe hypoglycaemia.

**Results:** In T2DM higher scores in CO are correlated with a lower mean HbA<sub>1c</sub>, R=-0.106[-0.504;-0.050], p<0.05. Personality traits or RES have no correlation to hypoglycaemia. Subgroup analysis of the highest and lowest scores of the personality traits showed that patients with higher scores in EX, OP and CO have a significantly (p<0.05) higher social status (EX 12.73 vs. 11.02 / OP 12.51 vs. 10.58 / CO 12.15 vs.10.46), less polyneuropathy, lower use of insulin (units/day) (EX 33.84 vs. 49.89 / OP 34.85 vs. 47.28 / CO 34.42 vs. 58.29), and higher scores for RES (EX 75.41 vs. 64.07 / OP 74.55 vs. 66.61 / CO 75.78 vs. 64.94) and lower scores for NE (EX 3.34 vs. 3.7 / OP 3.59 vs. 3.64 / CO 3.47 vs. 3.72). Patients with higher scores in NE have a higher BMI (35.27 vs. 32.69 kg/m<sup>2</sup>, p<0.05) and lower scores in EX, AG and RES (62.6 vs. 74.26, p<0.01). In T1DM no significant correlations could be identified comparing personality traits or RES and HbA<sub>1c</sub>, BMI and rates of hypoglycaemia. In subgroup analysis patients with higher scores in CO have a higher social status, 13.02 vs. 10.91, p<0.05, and patients with higher scores in NE have a lower social status, 11.5 vs. 13.6, p<0.05, and treatment satisfaction, 24.88 vs. 27.82, p<0.05. In both T1DM and T2DM higher scores in RES, and AG were positively correlated with the treatment satisfaction.

**Conclusion:** Apart from a weak correlation for CO and mean HbA<sub>1c</sub> in T2DM we found no significant influence of personality traits or RES on HbA<sub>1c</sub> and rates of hypoglycaemia. Higher RES and AG are positively associated with higher treatment satisfaction. Seemingly, psychic healthy persons are mostly sufficiently able to cope with diabetes related stress. It is known that participating in a structured treatment and education program equalised differences related to social status. A similar effect may occur with personality related differences as well.

## 953

**The ticking time bomb - fear of hypoglycaemia and its impact on diabetes control: baseline results from the ABACUS**K. Barnard<sup>1</sup>, D.A. Cavan<sup>2</sup>, R. Ziegler<sup>3</sup>, I. Cranston<sup>4</sup>, J. Ryder<sup>2</sup>, C. Vogel<sup>5</sup>, C.G. Parkin<sup>6</sup>, W. Koehler<sup>7</sup>, I. Vesper<sup>8</sup>, B. Petersen<sup>9</sup>, R.S. Wagner<sup>9</sup>;<sup>1</sup>University of Southampton, UK, <sup>2</sup>Royal Bournemouth Hospital, UK,<sup>3</sup>Diabetes Clinic for Children and Adolescents, Muenster, Germany, <sup>4</sup>QueenAlexandra Hospital, Portsmouth, UK, <sup>5</sup>Internistisches Facharztzentrum,Langen, Germany, <sup>6</sup>CGParkin Communications, Inc., Boulder City, USA,<sup>7</sup>baseline statistics GmbH, Mannheim, Germany, <sup>8</sup>Roche Diagnostics GmbH, Mannheim, Germany, <sup>9</sup>Roche Diagnostics, Inc., Indianapolis, USA.

**Background and aims:** Diabetes self-management is demanding and relentless, with prevalence of depression significantly higher for people with

both type 1 (T1DM) and type 2 (T2DM) diabetes. Fear of hypoglycaemia is a significant problem with a pervasive and detrimental effect on patients' abilities to self-manage their disease. However, such factors are not routinely addressed in clinical care, which can result in poor clinical and psychosocial outcomes. We explored the prevalence and impact of fear of hypoglycaemia in patients with poorly controlled diabetes.

**Materials and methods:** Baseline data were drawn from 116 subjects enrolled in the Automated Bolus Advisor Control and Usability Study (ABACUS), a large, prospective, randomised, multi-national study of poorly controlled ( $\text{HbA}_{1c} \geq 7.5\%$  /  $58 \text{ mmol/l}$ ), MDI-treated people with T1DM and T2DM. At screening, investigators administered the Hypoglycaemia Fear Survey (HFS-II), a validated measure of avoidance behaviours and worries specific to use of insulin.

**Results:** In this cohort with sub-optimal diabetes control, fear of hypoglycaemia was common, with 39.1% ( $n = 45$ ) of participants reporting that they "often" or "always" reduce their insulin dose to avoid hypoglycaemia; 34.2% ( $n = 39$ ) indicated that they performed self-monitoring of blood glucose (SMBG)  $\geq 6$  times per day as an avoidance strategy. Fear of hypoglycaemia within this population was correlated with increased problem areas in diabetes, higher mean blood glucose, increased depressive prevalence, and reduced diabetes treatment satisfaction. (Table 1)

**Conclusion:** These data suggest that fear of hypoglycaemia is highly prevalent and associated with poor glycaemic control and poor psychosocial health. These patients require management strategies that recognise and address this fear. Tools that minimise the risk of hypoglycaemia will likely increase confidence in achieving better control of diabetes; an automated bolus advisor may be one of those tools. This will be evaluated in the ABACUS.

Table 1. Correlation of fear of hypoglycaemia with other measures of clinical and psychosocial well-

Clinical/psychosocial measure	Correlation coefficients
Problem areas in diabetes	0.57
Mean blood glucose	0.39
Depressive disorders	0.35
Diabetes treatment satisfaction	-0.46

Clinical Trial Registration Number: NCT01460446

Supported by: Roche Diagnostics, Inc.

## 954

**Novel long-acting basal insulin analogue LY2605541 significantly reduces nocturnal hypoglycaemia and fear of hypoglycaemia compared to insulin glargine in patients with type 2 diabetes mellitus**

B. Curtis<sup>1</sup>, C. Shi<sup>2</sup>

<sup>1</sup>Global Health Outcomes, Eli Lilly and Company, Indianapolis, USA, <sup>2</sup>3 Statprobe, Detroit, USA.

**Background and aims:** The clinical impact of mild-moderate hypoglycemia (MH) is unclear. While physiologic manifestations are subtle, emotional impacts on the patient, reaction of the practitioner, and subsequent changes in their respective behaviors may lead to sub-optimal long-term outcomes. The aim of this analysis was to evaluate the impact of MH on patient behavior and fear of hypoglycemia using a validated patient-reported measure, the Adult Low Blood Sugar Survey (ALBSS).

**Materials and methods:** The basal insulin analog LY2605541 (LY) is a PE-Glylated insulin lispro designed to have a large hydrodynamic size which delays insulin absorption and reduces clearance, resulting in prolonged duration of action. In a 12-week, Phase 2, randomized, open-label, parallel study in patients with type 2 diabetes (T2DM), LY was compared to the basal insulin glargine (GL). The ALBSS is a patient-reported questionnaire, with items rated on a 5-point Likert scale (score 0-4) where a lower score indicates less impact of the item being measured. The behavior subscale has 15 items and the fear subscale 18 items. Patients completed the ALBSS at baseline, week 6, and week 12 of the study. Only patients who had baseline and at least 1 post-baseline measurement were included in the analyses. Treatment comparison was assessed using analysis of covariance (ANCOVA) model.

**Results:** At baseline (LY v GL) mean  $\pm$  SE FBG was  $8.16 \pm 0.17$  v  $7.77 \pm 0.22 \text{ mmol/L}$  and  $\text{HbA}_{1c}$  was  $7.7 \pm 0.1$  v  $7.8 \pm 0.1\%$ . At week 12, LY v GL resulted in similar mean fasting blood glucose ( $p = .433$ ) and mean  $\text{HbA}_{1c}$  ( $p = .279$ ) values. Intra-day blood glucose (BG) variability, as measured by

8-point self-monitored BG standard deviation, was reduced with LY ( $1.89 \pm 0.06$  v  $2.16 \pm 0.11 \text{ mmol/L}$ ,  $p = .031$ ). Adjusting for baseline nocturnal hypoglycemia, LY pts had a 48% rate reduction in nocturnal hypoglycemia events compared to GL ( $p = .021$ ). The mean scores of the behavior, fear, and total ALBSS score were not statistically different at baseline. The mean ALBSS fear score at week 12 was 6.6 (SD: 9.1) and 10.0 (SD: 15.8) for the LY and GL groups respectively, with an LS mean difference (LY-GL) of -2.3 (-4.0, -0.7 [ $p=.022$ ]). The mean ALBSS total score at week 12 was 13.0 (SD: 15.0) and 16.5 (SD: 22.5) for the LY and insulin GL groups respectively, with an LS mean difference (LY-GL) of -3.4 (-5.9, -0.9, [ $p=.026$ ]).

**Conclusion:** In patients with Type 2 diabetes, the novel long-acting basal insulin LY2605541 significantly reduced the baseline-adjusted rate of nocturnal hypoglycemia and patient fear of hypoglycemic events. The authors hypothesize that change in the ALBSS behavior sub-scale did not materialize due to the nature of a protocol-driven clinical trial, in which patients are more likely to comply with treatment recommendations. Further Phase 3 studies are underway to confirm these findings and to evaluate the impact on longer term health outcomes.

Clinical Trial Registration Number: NCT01027871

Supported by: Eli Lilly and Co

## 955

**GAPP2™: Global survey finds one in eight patients intentionally reduce basal insulin doses to avoid hypoglycaemia in the last month**

M. Brod<sup>1</sup>, M. Peyrot<sup>2</sup>, A. Rana<sup>3</sup>, A.H. Barnett<sup>4</sup>

<sup>1</sup>The Brod Group, Mill Valley, USA, <sup>2</sup>Sociology Department, Loyola University, Baltimore, USA, <sup>3</sup>Medical Affairs, Novo Nordisk, Copenhagen, Denmark, <sup>4</sup>Heart of England NHS Foundation Trust and University of Birmingham, UK.

**Background and aims:** There remain gaps in understanding about patient insulin-taking behaviour and why patients may miss doses. Questions about insulin-taking behaviour were therefore included in a large global survey (GAPP2™) of type 2 diabetes (T2DM) patients and healthcare professionals (HCPs) (primary care, diabetes specialists and diabetes nurses/educators) carried out to gain new insights into the challenges of insulin treatment and their impact on diabetes management.

**Materials and methods:** The survey was performed in six countries: USA, Canada, Japan, UK, Germany and Denmark. HCPs involved in diabetes management and T2DM patients (diagnosed at  $\geq 40$  years) receiving insulin treatment were identified from online research panels with over 6.5 million members.

**Results:** Data are presented on insulin-taking behaviour of 3042 T2DM patients using insulin analogue (IA), and experiences of 1653 HCPs. Patients reported irregular dosing of the basal insulin (BI). 48% had missed a dose, 51% had mis-timed by  $> 2$  hours and 38% had reduced a dose of BI. In the previous 30 days patients reported missing (22%: mean  $3 \pm 0.16$ ), mis-timing (24%: mean  $4.2 \pm 0.21$ ) or reducing (14%: mean  $4.2 \pm 0.24$ ) a dose of their BI. During this period, patients reported missing (17%), mis-timing (27%) or reducing (27%) their BI dose 5 times or more in a given month. This was greater than or approximately equivalent to the magnitude of dosing irregularities that HCPs reported as being significant enough to have a clinical impact on glucose control (for BI only patients:  $4.4 \pm 0.1$  missed,  $5.6 \pm 0.18$  mis-timed, or  $5.1 \pm 0.13$  reduced BI doses and for basal bolus patients:  $4.5 \pm 0.1$ ,  $5.6 \pm 0.18$ ,  $4.8 \pm 0.12$  respectively). On the last occasion that patients had missed, mis-timed or reduced their dose of BI, 13%, 17% and 77% respectively had done so intentionally. The most commonly cited reasons for this behaviour were low blood sugar levels and reduction in hypoglycaemia risk. Most patients recognised the clinical importance of irregular dosing. 63% of patients thought missing BI doses would have a negative impact on their long-term health and 37% reported they would feel guilty if they missed a BI dose. Only 10% of patients reported that they downplayed the number of missed doses to their HCPs, however, HCPs thought around 30% of patients downplayed missed doses.

**Conclusion:** Some patients intentionally dose their BI irregularly citing reduction in hypoglycaemia risk or low blood sugar as reasons for this behaviour. A proportion of patients undertake mis-dosing behaviour at a frequency that would be of concern to the HCPs despite an appreciation of the potential long-term consequences for their health. Further research is needed to focus on interventions which address the reasons for regular and intentional poor insulin-taking behaviour highlighted by our results.

Most common reasons for intentionally missing/mis-timing/reducing doses of basal insulin

	Missing a dose	Taking a dose > 2 hr earlier or later than prescribed	Reducing a dose
Total (n)	178	237	856
Blood sugar low	38%	21%	50%
Blood sugar high	7%	12%	3%
Reduced risk of hypoglycaemia	39%	22%	43%
Advised by a HCP	8%	3%	20%
Changed eating pattern	21%	30%	16%
Ran out/running low on insulin	17%	6%	9%
Skipped a meal	18%	14%	9%
Exercised recently	9%	8%	7%
Social situation	11%	20%	2%

(multiple choice responses permitted)

Supported by: The GAPP2™ surveys were supported by a grant from Novo Nordisk

## 956

### Assessing the relationship between the effect of glycaemic control and avoided symptomatic hypoglycaemia on quality of life in the management of type 2 diabetes

V. Foos<sup>1</sup>, P. McEwan<sup>2</sup>, A. Lloyd<sup>3</sup>, J. Palmer<sup>4</sup>, M. Lamotte<sup>5</sup>, D. Grant<sup>6</sup>;

<sup>1</sup>IMS Health, Basel, UK, <sup>2</sup>Swansea University, Swansea, UK, <sup>3</sup>IMS Consulting Group, London, UK, <sup>4</sup>IMS Health, Basel, Switzerland, <sup>5</sup>IMS HEOR, Vilvoorde, Belgium, <sup>6</sup>IMS Health, London, UK.

**Background and aims:** Achieving optimal blood glucose control minimizes long-term diabetes related complications. Hypoglycaemia represents a potential barrier to achieving optimal glycaemic control and also impacts directly on costs and health utility; furthermore, the hypoglycaemia profile of an intervention is potentially subject to less compound discounting if evaluated as an initial therapy. The objective of this study was to undertake an equilibrium analysis to identify the level of HbA<sub>1c</sub> reduction required to achieve the same quality of life benefit as achieved by avoiding events of non-severe symptomatic hypoglycemia (NSHE).

**Materials and methods:** This study used the Core Diabetes Model (CDM), a validated and established diabetes model, to explore the relationship between the avoidance of NSHE, HbA<sub>1c</sub> reduction and quality-adjusted life expectancy (QALE). Scenario analysis (SA) comparing typical therapeutic profile compared Treatment A versus Treatment B assuming 1) differential rates of NSHE ranging from 25 to 250 events per 100 patient years and no HbA<sub>1c</sub> effect and 2) an increasing difference in HbA<sub>1c</sub> ranging between 0.1% and 1% points (0.1% increments) with no effect on NSHE. The model was run over a lifetime and benefits were discounted at 3.0%. Patient baseline characteristics were based on NHANES population with a mean baseline A1c of 7.4%. Incremental QALE was observed using alternative assumptions for the disutility of symptomatic hypoglycemia based on published data (base case: -0.0052; SA: -0.0029, -0.0107, -0.0184).

**Results:** The relationship between rates of NSHE and incremental QALE was linear ( $R^2 > 0.9999$ ). The QALE gain associated with avoiding 1 NSHE per patient per year was 0.06; incremental QALE per event avoided changed in scenario analyses to 0.035, 0.124 and 0.212 quality adjusted life years for assumed disutilities of -0.0029, -0.0107 and -0.0184, respectively. The impact of HbA<sub>1c</sub> reduction on QALE gain ranged from 0.0112 to 0.1171 for changes between 0.1% and 1.0% points, respectively (also exhibiting a linear relationship;  $R^2 = 0.9922$ ). The HbA<sub>1c</sub> reduction required to achieve equivalent QALE benefit as the avoidance of one NSHE per patient per year was 0.54% in the base case analysis (NSHE disutility of -0.0052) and 0.33%, 1.08% and 1.84% in SA for NSHE disutilities of 0.0029, -0.0107 and -0.0184, respectively.

**Conclusion:** HbA<sub>1c</sub> change and reduced NSHE are key drivers when diabetes interventions are compared in cost effectiveness analyses. This analysis is noteworthy as it demonstrates the significant contribution to QALE exhibited by the avoidance of NSHE; particularly in comparison with levels of HbA<sub>1c</sub>

change typically associated current anti-hyperglycaemic agents. The avoidance of NSHE is at least as powerful a driver of QALE as lowering HbA<sub>1c</sub>.

## 957

### Health status in people with type 2 diabetes on basal-oral therapy is significantly improved with insulin degludec vs insulin glargine

J. Jendle<sup>1</sup>, N. Freemantle<sup>2</sup>, L. Meneghini<sup>3</sup>, T.E. Christensen<sup>4</sup>, M.L. Wolden<sup>4</sup>, R.E. Ratner<sup>5</sup>;

<sup>1</sup>Endocrine and Diabetes Center, University of Orebro, Sweden, <sup>2</sup>University College London, UK, <sup>3</sup>University of Miami Miller School of Medicine, USA, <sup>4</sup>Novo Nordisk, Søborg, Denmark, <sup>5</sup>MedStar Health Research Institute, Hyattville, USA.

**Background and aims:** Health status plays a critical role in therapy adherence in people with diabetes. Insulin degludec (IDeg) is a new-generation, ultra-long-acting basal insulin, forming soluble multi-hexamers following subcutaneous injection, achieving a stable time-action profile >24 hrs. This meta-analysis of patient level data from three open-label, randomised trials of 26 or 52 weeks duration compared the effect of IDeg and insulin glargine (IGlar) on health status in people with type 2 diabetes on basal-oral therapy.

**Materials and methods:** We assessed glycaemic control via HbA<sub>1c</sub> and fasting plasma glucose (FPG) concentrations, hypoglycaemia, defined as plasma glucose <3.1 mmol/L, and health-related quality of life, using the Medical Outcomes Study Short Form 36 (SF-36). Insulin-naïve patients received IDeg (n=1290) or IGlar (n=632) once daily, in combination with oral antidiabetic drugs. Statistical analysis was performed using a generalised linear model with treatment, trial, antidiabetic therapy at baseline, gender, region, age and relevant baseline values as explanatory variables.

**Results:** At baseline, mean age was 58.6 yrs, HbA<sub>1c</sub> 67 mmol/mol (8.3%), FPG 9.4 mmol/L and BMI 30.0 kg/m<sup>2</sup>. In all three trials, IDeg was confirmed as non-inferior to IGlar based on HbA<sub>1c</sub>. In each of the trials comprising the meta-analysis, FPG and confirmed overall and nocturnal (00:01-05:59 hrs) hypoglycaemia were all numerically or significantly lower with IDeg vs IGlar. At study end, overall physical component score was significantly higher (better) with IDeg vs IGlar (+0.66 [95% CI: 0.04; 1.28]), largely due to a difference (+1.10 [95% CI: 0.22; 1.98]) in the bodily pain domain score. In the mental domains, vitality was significantly higher with IDeg vs IGlar (+0.81 [95% CI: 0.01; 1.59]). The remaining SF-36 domains had scores in favour of IDeg, but were not significantly different between insulins.

**Conclusion:** Compared with IGlar, IDeg leads to improvements in both mental and physical health status for people with type 2 diabetes on basal-oral therapy.

Supported by: Novo Nordisk

## 958

### Binge-eating disorder in adults with newly diagnosed type 2 diabetes - baseline data from the SOUL-D study

H. Akram<sup>1</sup>, C. Kan<sup>1</sup>, S. Amiel<sup>2</sup>, K. Winkley<sup>1</sup>, K. Ismail<sup>1</sup>;

<sup>1</sup>Psychological Medicine, <sup>2</sup>Diabetes Research Group, Kings College London, UK.

**Background and aims:** The relationship between eating disorders and type 2 diabetes (T2DM) is poorly understood. The published prevalence of binge-eating disorder (BED) ranges from 1.4 to 25.6% among individuals with T2DM. The present study aims to ascertain the prevalence of BED among participants from the South London Diabetes (SOUL-D) Study and its impact on glycaemic control.

**Materials and methods:** SOUL-D is a community based prospective cohort study examining a range of biological, psychological and social factors in people with newly-diagnosed T2DM. The Eating Disorders Diagnostic Scale (EDDS) was used to screen for BED and the Patient Health Questionnaire (PHQ-9) for depression. BMI and HbA<sub>1c</sub> were also measured.

**Results:** From those who completed the EDDS questionnaire (n=1154), 20 reached full BED and 4 subthreshold BED diagnosis, representing 2.1% of the total sample. In comparison to subjects without BED, subjects with BED were significantly younger (mean(SD):50.9(12.6) vs. 55.8(11.5),  $p=0.032$ ), more likely to be female (83.3% vs. 42.3%,  $p<0.001$ ), in full-time employment (70.8% vs. 46.0%,  $p=0.016$ ) and less likely to be married (29.2% vs. 49.8%,  $p=0.046$ ). They also had higher BMI (37.9kg/m<sup>2</sup>(9.87) vs. 31.5kg/m<sup>2</sup>(6.3),  $p<0.001$ ), poorer glycaemic control (7.53%(1.56) vs. 6.97%(1.45),  $p=0.047$ ) and more depressive symptoms (9.27(7.34) vs. 4.23(5.22),  $p<0.001$ ).



**Conclusion:** There is a significant association between BED and worse metabolic and psychological outcomes in subjects with newly diagnosed T2DM. The finding underscores the relevance of assessing and addressing disordered eating behaviours in patients with T2DM, in order to improve biomedical and psychological outcomes.

*Clinical Trial Registration Number:* RDLSLB410 REC 08/H0

*Supported by:* NIHR

## 959

### Does personality affect social status in patients with type 1 and type 2 diabetes?

F. Zillich<sup>1</sup>, W.C. Keweloh<sup>2</sup>, K. Wick<sup>1</sup>, N. Müller<sup>2</sup>, C. Kloos<sup>2</sup>, T. Lehmann<sup>3</sup>, G. Wolf<sup>2</sup>, U.A. Müller<sup>2</sup>;

<sup>1</sup>Institute of Psychosocial Medicine and Psychotherapy, <sup>2</sup>Endocrinology and Metabolic Diseases, Internal Medicine III, <sup>3</sup>Institute of Medical Statistics, Information Sciences and Documentation, University Hospital, Jena, Germany.

**Background and aims:** Patients with low social status showed worse HbA<sub>1c</sub> at the beginning of diabetes therapy in a tertiary care center but differences in therapy outcome related to social status were equalised while participating in structured therapy and education programs. We assessed personality traits neuroticism (NE), extraversion (EX), conscientiousness (CO), agreeableness (AG), openness (OP) as well as resilience (RES) as influencing factors on social status and therapy success or failure.

**Materials and methods:** We conducted a cross-sectional study of patients with type 1 (T1DM) and type 2 diabetes (T2DM) in a tertiary care centre in Germany during a period of three month. We assessed the short version of the Big Five Inventory (BFI-S), resilience scale (RS-13), treatment satisfaction (DTSQ standard) and social status in 669 patients (162 T1DM, age 52.17, duration of diabetes 20.05 years, HbA<sub>1c</sub> 7.51%, BMI 26.6 kg/m<sup>2</sup>, social status 12.67, 507 T2DM, age 67.43, duration of diabetes 15.4 years, HbA<sub>1c</sub> 7.31%, BMI 32.9 kg/m<sup>2</sup>, social status 11.65). Social status was measured by education level, highest professional position and household net income (total score 3–21). Quality of diabetes care was measured by HbA<sub>1c</sub>, blood pressure, BMI and rates of serious and severe hypoglycaemia.

**Results:** In T2DM differences in social status are causal for 5.8% of the differences in mean HbA<sub>1c</sub>.  $R=0.283$  [−0.081; 0.018],  $p<0.01$ . Higher social status goes along with higher scores for EX,  $R=0.347$  [0.277; 1.642];  $p<0.01$ , CO,  $R=0.434$  [0.058; 1.764],  $p<0.05$ , OP,  $R=0.422$  [0.986; 2.643],  $p<0.01$  and RES,  $r=0.172$ ,  $p<0.01$  and lower scores for NE,  $r=-0.102$ ,  $p<0.05$ . Subgroup analysis of the highest and lowest scores of the personality traits showed similar results. In T1DM higher social status are correlated to higher scores in OP,  $R=0.237$  [0.942; 4.305],  $p<0.01$  and lower scores in NE,  $R=-0.172$  [−2.655; 0.371],  $p<0.01$ . In subgroup analysis higher scores in CO were correlated to a higher social status, 13.02 vs. 10.91,  $p<0.05$  and significantly higher scores for RES, 75.02 vs. 61.48,  $p<0.01$  and EX, 3.22 vs. 2.86,  $p<0.01$ . Higher scores in NE are related to a lower social status, 11.5 vs. 13.6,  $p<0.05$  and to lower scores of RES, 73.45 vs. 62.71,  $p<0.01$ .

**Conclusion:** In both T1DM and T2DM higher social status means better therapy outcome. Higher social status is especially related to higher scores in conscientiousness and lower scores in neuroticism in T1DM and T2DM. Therefore people who are i.e. nervous, anxious, hostile, vulnerable or depressed might have lower social status when connected with treatment of diabetes. An association with depression or other psychiatric diseases should be examined. Resilience seems to correlate significantly with social status. Whether social status has significant influence on resilience or resilience influences social status remains to be clarified.

## 960

### Differences in social network and social support in people with type 1 and type 2 diabetes and the role of socioeconomic status

N.F. Hempler, L.E. Joensen, I. Willaing;

Steno Health Promotion Center, Steno Diabetes Center, Gentofte, Denmark.

**Background and aims:** A good social network and social support is associated with fewer psychosocial problems and better self-management behaviours in people with diabetes. People with type 1 and type 2 diabetes have different characteristics; however, little is known about differences in social network and social support in relation to diabetes type, and how socio-demographic factors might influence possible differences. We explored 1) differences in so-

cial network and social support between people with type 1 and type 2 diabetes and 2) whether differences were influenced by education level.

**Materials and methods:** We compared cross-sectional survey data from three populations: people with type 2 diabetes (N=1,084) and type 1 diabetes (N=2,426) from a specialist diabetes clinic and people with type 2 diabetes from a web panel (N=1,491). The web panel consisted of a representative sample of Danish people with type 2 diabetes and using the Internet. Social network was measured as contacts to friends and family, whereas social support was measured as the ability to get help from the social network in case of severe illness. All analyses were adjusted for age and cohabitation. Using logistic regression models we calculated the relative excess risk due to interaction (RERI), which indicates existing moderating effects of education.

**Results:** Our findings show that people with type 2 from the diabetes clinic (OR 2.01; 95% CI 1.57–2.59) and the web panel (OR 2.23; 95% CI 1.78–2.79) more often reported little contact with family, compared to people with type 1 diabetes. A similar pattern was observed regarding contact with friends. Furthermore, people with type 2 diabetes from the diabetes clinic (OR 1.44; 95% CI 1.44–2.08) and the web panel (OR 1.85; 95% CI 1.58–2.17) more often reported that they could not count on getting help in case of severe illness, compared with people with type 1 diabetes. The influence of education on social support and social network were more pronounced among people with type 2 compared to people with type 1. No notable differences were observed when analyses were stratified for sex.

**Conclusion:** In conclusion, people with type 2 diabetes have poorer social networks and less social support than people with type 1 diabetes. The findings indicate that differences in social network and social support to a larger extent were influenced by educational status in people with type 2 diabetes compared to people with type 1. Translating our findings into interventional practice, we suggest that interventions aiming at improving and establishing social relations and social support should be particularly emphasised in people with type 2 diabetes with low education.

## 961

### How do patients' preferences compare to the present spectrum of diabetes research?

S. Arnolds<sup>1</sup>, S. Heckermann<sup>1</sup>, P.T. Sawicki<sup>2</sup>;

<sup>1</sup>Profil Institute for Metabolic Research, Neuss, <sup>2</sup>Institute for Health Economics and Clinical Epidemiology, Medical Faculty of the University of Cologne, Germany.

**Background and aims:** It is unclear how current topics in diabetes research correspond to the research patients would like to see done. Thus, it was our aim to assess patients' preferences in diabetes research and compare them to the spectrum of scientific topics as presented during recent annual meetings of the European Association for the Study of Diabetes (EASD).

**Materials and methods:** Four experienced, scientifically active diabetologists divided the entire spectrum of diabetes research into nine main topics (table) - defined by consensus. The completeness of the proposed fields was checked by testing whether each and every abstract published during the EASD meeting in 2008 was covered by a defined scientific field. In order to obtain an as far as possible unselected sample of interested patients a questionnaire was published in a popular weekly news magazine, accompanied by an article inviting patients with diabetes and their relatives to express their preferences in diabetes research. They were asked to classify each of the nine prespecified scientific fields using a scale from 1 to 9 (1=least, 9=very important) and to decide which of the nine areas was of top priority. The status of the current spectrum of diabetes research was assessed by means of recent EASD abstracts. Two reviewers independently allocated all EASD abstracts published in 2010 and 2011 to one of the nine prespecified research fields. In case of disagreement a third reviewer was consulted.

**Results:** The main results are shown in the table. The questionnaire was answered by 652 patients with diabetes, 205 relatives and 61 other persons interested in the disease. The most important diabetes research fields from the patients' perspective were "development, pathophysiology and prevention of diabetes" (25.6%), "transplantation and cell therapy" (19.4%) and "blood glucose measurement and artificial pancreas" (16.5%), whereas "extreme blood values including hypoglycaemia and hyperglycaemia" was considered least important (2.4%). In 2010 and 2011 2645 EASD abstracts were published. The most often covered topic of current research was "development, pathophysiology and prevention of diabetes" (46.3%), followed by "diabetes complications in man" (17.5%), whereas "transplantation and cell therapy" had the lowest number of abstracts (0.7%).

**Conclusion:** The priorities and preferences of patients with diabetes and their relatives regarding the main diabetes research fields may differ when compared to current scientific activity. Institutions, sponsors and research foundations may consider involving patients with diabetes and their relatives more often in the weighting and selection of research topics.

Research preferences of survey participants versus EASD abstracts in 2010 and 2011

Field of research	Importance of research field* Mean score (SD) from 1=least important to 9=very important	Top priority field of research* n (%) of all responses	EASD 2010 + 2011 abstracts: assignment to field of research n (%)**	EASD 2010 + 2011 abstracts: Commercial sponsorship n (%)***
1) Development, pathophysiology and prevention of diabetes	8.14 (1.54)	235 (25.60)	1225 (46.31)	114 (9.31)
2) Transplantation and cell therapy	6.86 (2.36)	178 (19.39)	19 (0.72)	0 (0)
3) Non-pharmaceutical therapy	6.82 (2.53)	81 (8.82)	163 (6.17)	18 (11.04)
4) Insulin therapy	7.25 (2.08)	63 (6.86)	131 (4.95)	98 (74.81)
5) Blood-glucose lowering therapy without insulin	7.08 (2.36)	110 (11.98)	247 (9.34)	213 (86.23)
6) Extreme blood values including hypoglycaemia and hyperglycaemia	7.14 (2.08)	22 (2.40)	38 (1.44)	12 (31.58)
7) Diabetes complications in man	7.83 (1.67)	42 (4.58)	463 (17.50)	71 (15.33)
8) Special situations, training, psychology, treatment- and care structures	7.23 (2.26)	36 (3.92)	278 (10.51)	33 (11.87)
9) Blood-glucose measurement, devices and artificial pancreas	7.71 (1.82)	151 (16.45)	81 (3.06)	46 (56.79)

\*as assigned by the 918 survey participants; \*\*of all accepted abstracts; \*\*\*of accepted abstracts in the respective field of research

## PS 079 Psychological aspects II

962

### Poor well-being and psychosocial risk factors among adolescents and young adults with diabetes: prevalence and association to metabolic control

K. Lange<sup>1</sup>, S. Enax<sup>1</sup>, K.-M. Röhlver<sup>2</sup>

<sup>1</sup>Medical Psychology, Hannover Medical School, Hannover,

<sup>2</sup>Diabeteszentrum, Christliches Krankenhaus, Quakenbrück, Germany.

**Background and aim:** Previous studies have suggested that psychological factors are important influences affecting the care and management of diabetes in adolescence. The study “Lebenschancen (life chances) with diabetes 2011” focuses on the prevalence of psychosocial risk factors, physical and psychosocial well-being and patients’ needs in the subsequent phases of transition to internal medicine and in young adulthood.

**Methods:** The participants of a diabetes camp for 16-25 year old people from all over Germany (CAMP D) were invited to fill in a questionnaire on their physical status (HbA1c, co morbidity, diabetes complication), emotional well-being (WHO-5 and HADS questionnaire) and diabetes related distress (PAID). In addition clinical and socioeconomic characteristics and satisfaction with main aspects of diabetes care (Likert scale: 1 optimal - 6 bad) were assessed.

**Results:** Data were received from 368 (91%) of the participants of CAMP D (64.5% female, mean age 20.2±2.6 y, mean diabetes duration 8.5±5.5 y, 45.7% CSII and 54.3% MDI). Late complications were diagnosed in 6.8% of the patients, but additional 13% didn’t know about it. 19.8% of the participants were affected by a second chronic disease (e. g. 2% asthma, 2% celiac, 8% hypo/hyperthyroidism). Mean HbA1c was 8.4±1.7% with 26% of the patients in insufficient control (HbA1c > 9%). While diabetes related distress (PAID) was rated low (22.9±14.5 (min 0 - max 80)), overall emotional well-being was severely impaired in 36.6% (WHO-5 sum score <13). 11% of participants showed (sub-) clinical symptoms of an anxiety disorder (HADS-A) and 4% symptoms of depression (HADS-D). 19.2% reported of former or current psychotherapy due to depression (10.1%), eating disorder (4.1%), anxiety (2.2%) or alcohol abuse (2.2%). In contrast to high satisfaction with medical care and diabetes education (mean scores: 2.1±1.1 / 2.3±1.3), satisfaction with psychosocial care was rated low (mean score: 3.0±1.5) by 230 participants. The remaining 35.8% young people stated that no psychosocial care was available. Emotional well-being (WHO-5) was associated with diabetes distress (r=-0.38), satisfaction with diabetes care (r=-0.25), gender (female < male), educational level (lower < higher) and family structure (parents living apart < living together). Participants with psychological co morbidity reported higher HbA1c than those without (9.1±1.9% vs. 8.2±1.6%) and lower well-being (12.5±4.7 vs. 14.3±5.0) (each p < 0.01). There was no association between type of insulin therapy (CSII or MDI) and HbA1c or emotional well-being (each p > 0.1).

**Conclusion:** More than 1/3 of the young people with diabetes reported of substantial impaired emotional well-being and 19% of former or current indication for psychotherapy. Psychosocial diabetes care was missed by more than a 1/3 of the young participants. On the background of the close association between metabolic control, emotional well-being, burden due to somatic and psychological co morbidities and psychosocial risk factors, better structures for integrated psychological screening and psychosocial care for young adults with diabetes are necessary.

Supported by: Deutsche Diabetes Stiftung and Novo Nordisk

## 963

### Adolescent girls with type 1 diabetes have poorer health perception and quality of life than the general population. A study in 18 countries on 95,870 adolescents

H.M.C. Hoey<sup>1</sup>, M. Trab Damsgaard<sup>2</sup>, P. Due<sup>2</sup>, C. de Beaufort<sup>3</sup>, H. Mortensen<sup>4</sup>, T.C. Skinner<sup>5</sup>, H.-J. Aanstoot<sup>6</sup>, L. Castano<sup>7</sup>, K.J. Robertson<sup>8</sup>, P. Swift On behalf of The Hvidoere Study Group<sup>9</sup>;

<sup>1</sup>Dept Paediatrics, University of Dublin, Trinity College, Ireland, <sup>2</sup>National Institute of Public Health, Copenhagen, Denmark, <sup>3</sup>Diabetes and Endocrine Care CP, Clinique Pédiatrique/CHL, Luxembourg, <sup>4</sup>E Herlev Hospital, Faculty of Health Science, Copenhagen, Denmark, <sup>5</sup>Rural Clinical School, Tasmania, Australia, <sup>6</sup>Diabetes Center for Paediatric and Adolescent Diabetes Care and Research, Rotterdam, Netherlands, <sup>7</sup>Hospital de Cruces, Bilbao, Spain, <sup>8</sup>Royal Hospital for Sick Children, Glasgow, UK, <sup>9</sup>Children's Hospital, Leicester Royal Infirmary, UK.

**Background and aims:** The Health Behaviour in School-Age Children study (HBSC) is a cross-national study, of 43 countries from Europe and North America and investigates health and health behaviour of adolescents. The Hvidoere Study Group (HSG) has shown that health perception and Quality of Life (QOL) of adolescents with type 1 diabetes (T1D) is poorer in girls than in boys and that good QOL and health perception are associated with better metabolic control. The aim of this study was to investigate whether QOL and health perception in adolescents with diabetes differs from their healthy peers.

**Materials and methods:** Adolescents aged 11, 13 and 15 years from 18 countries where data from both the HBSC and the HSG studies are available were studied (94,387 from the HBSC study and 1,483 with T1D from the HSG). Measures of QOL included life satisfaction (Cantril ladder) (score 0–10; high score indicates good life satisfaction) and health perception (rated excellent, good, fair or poor).

**Results:** Adolescents with diabetes particularly the older girls reported significantly poorer health perception than the general population and also lower than that of boys with diabetes. Health perception rated fair or poor - 11yrs: boys 10.7% vs HBSC 9.0% ( $p=0.056$ ), girls 18.7% vs HBSC 10.4% ( $p=0.0005$ ); -13 yrs: boys 21.3% vs HBSC 10.8% ( $p<0.0001$ ), girls 24.9% vs HBSC 15.6% ( $p<0.0001$ ); -15 yrs: boys 19% vs HBSC 12.1% ( $p=0.0006$ ), girls 35.69% vs HBSC 20.34% ( $p<0.0001$ ). Girls with diabetes reported less life satisfaction score by age than boys with T1D and also lower than that of the general population (HBSC): mean scores -11yrs: boys 8.08 vs HBSC 8.17, girls 7.74 vs HBSC 8.21; -13 yrs: boys 7.60 vs HBSC 7.83, girls 7.37 vs HBSC 7.56; and at 15 yrs boys 7.54 vs HBSC 7.52, girls 7.07 vs HBSC 7.17).

**Conclusion:** Adolescent girls with T1D have poorer health perception and QOL than their healthy peers. Good health perception and QOL are associated with better metabolic control and less perceived burden for the parents. Therefore good diabetic control should be promoted as a quality of life issue which should be audited yearly. Specific attention should be given to the management of adolescents with T1D, particularly girls, as they are very vulnerable and require additional resources.

*Supported by: Novo Nordisk*

## 964

### Self-reported chronic generic and diabetes-specific quality of life of 11- to 21-year olds with early-onset type 1 diabetes

A. Stahl<sup>1</sup>, K. Lange<sup>2</sup>, K. Straßburger<sup>1</sup>, K. Castillo<sup>1</sup>, C. Bächle<sup>1</sup>, T. Meissner<sup>3</sup>, R.W. Holl<sup>4</sup>, G. Giani<sup>1</sup>, J. Rosenbauer<sup>1</sup>, in cooperation with the German Pediatric Surveillance Unit (ESPED) and the DPV-Science initiative;

<sup>1</sup>Institute for Biometry and Epidemiology, German Diabetes Centre, Düsseldorf, <sup>2</sup>Medical Psychology Unit, Hannover Medical School, <sup>3</sup>University Hospital Düsseldorf, <sup>4</sup>Institute of Epidemiology and Medical Biometry, University of Ulm, Germany.

**Background and aims:** A major goal of diabetes care - especially in young patients with early-onset type 1 diabetes (T1DM) - is to achieve near-normal glycaemic control and unimpaired quality of life (QoL). This study aimed to analyse risk factors of impaired QoL in patients with early-onset T1DM and at least 10 years diabetes duration.

**Materials and methods:** Inclusion criteria for the nationwide questionnaire survey conducted in Germany 2009–2011 were T1DM onset occurring from 0 to 4 years of age during the years 1993–1999. QoL was assessed by means of the DISABKIDS self-report questionnaires: 12-item chronic generic module, diabetes-specific impact and treatment scales (scales 0–100, higher values

indicate better QoL). Analyses were performed with multivariable regression models. Results are reported as percentages, means and standard deviations (SD), or adjusted mean differences between groups (regression coefficients ( $\beta$ )) and standard errors (SE).

**Results:** Survey participants were 840 11- to 21-year olds with T1DM (50.6% boys, 16.3 (2.3) years of age, age at onset 3.0 (1.2) years, diabetes duration 13.3 (2.0) years, HbA1c 8.3 (1.4) %, 46.9% CSII). Poor glycaemic control was the strongest risk factor for impaired QoL (HbA1c  $>9.0$  vs.  $\leq 7.5$ :  $\beta_{\text{Generic}} = -9.1$  (1.5),  $\beta_{\text{Impact}} = -15.5$  (1.9),  $\beta_{\text{Treatment}} = -21.4$  (2.6), all  $p<0.001$ ; HbA1c  $>7.5\text{--}\leq 9.0$  vs.  $\leq 7.5$ :  $\beta_{\text{Generic}} = -5.7$  (1.2),  $\beta_{\text{Impact}} = -8.3$  (1.4),  $\beta_{\text{Treatment}} = -11.9$  (2.2), all  $p<0.001$ ) followed by impaired treatment satisfaction (poor vs. very good:  $\beta_{\text{Generic}} = -8.3$  (1.7),  $\beta_{\text{Impact}} = -9.2$  (2.0),  $\beta_{\text{Treatment}} = -14.9$  (2.9), all  $p<0.001$ ; good vs. very good:  $\beta_{\text{Generic}} = -2.7$  (1.2),  $\beta_{\text{Impact}} = -3.6$  (1.5),  $\beta_{\text{Treatment}} = -6.4$  (2.1), all  $p<0.05$ ). Hypoglycaemia during the last 12 months and therapy without CSII were also associated with impaired QoL (hypoglycaemia last month or week vs. never:  $\beta_{\text{Generic}} = -4.4$  (1.4),  $\beta_{\text{Impact}} = -3.8$  (1.7),  $\beta_{\text{Treatment}} = -6.5$  (2.4), all  $p<0.05$ ; CSII no vs. yes:  $\beta_{\text{Generic}} = -3.6$  (1.1),  $\beta_{\text{Impact}} = -5.2$  (1.3),  $\beta_{\text{Treatment}} = -4.9$  (1.9), all  $p<0.01$ ). Lower socio-economic status was associated with lower scores on two scales (low and middle vs. high:  $\beta_{\text{Generic}} = -4.0$  (1.1),  $\beta_{\text{Impact}} = -3.7$  (1.4), all  $p<0.01$ ). Overweight status and prior hospitalisation were each associated with poorer scores on one scale (overweight vs. under- and normal weight:  $\beta_{\text{Impact}} = -6.1$  (1.8),  $p=0.001$ ; hospitalisation during last 12 months yes vs. no:  $\beta_{\text{Generic}} = -3.1$  (1.3),  $p=0.017$ ). Older age was associated with better ratings on the impact scale (18–21y / 14–17y vs. 11–13y:  $\beta_{\text{Impact}} = 7.1$  (2.0) / 4.9 (1.7), all  $p<0.01$ ). Gender was not associated with QoL.

**Conclusion:** The results underline the close relationship between QoL and therapy (including treatment satisfaction, metabolic control, therapeutic regimen and hypoglycaemic episodes) among children, adolescents and young adults with early-onset and long duration T1DM.

*Supported by: Competence network Diabetes mellitus (support code 01GI0802)*

## 965

### The impact of type 2 diabetes on emotional state and quality of life

J. Ceponis<sup>1</sup>, L. Lasaitis<sup>2</sup>;

<sup>1</sup>Department of Endocrinology, <sup>2</sup>Institute of Endocrinology, Lithuanian University of Health Sciences, Kaunas, Lithuania.

**Background and aims:** The aim of the study was to analyze the impact of the course of disease on emotional state and quality of life (QoL) in patients with type 2 diabetes mellitus (T2DM) and outline possible gender-specific differences.

**Materials and methods:** A total of 218 patients (118 women and 100 men), aged 40–78 (mean  $59.5\pm 9.4$  years) were asked to complete Profile of Mood State (POMS) and WHO BREF Quality of Life (QoL) questionnaires. Data were grouped by diabetes duration (under 5 years, 5–10 years,  $>10$  years), quality of glucose control (HbA1c values of  $<6.5\%$ ,  $6.5\text{--}8\%$ , and  $>8\%$ ) and number of complications (0, 1,  $\geq 2$ ). Values were analyzed for both genders separately and results were compared.

**Results:** The 5–10 yrs. T2DM duration group demonstrated the most favorable POMS and QoL scores, however significance was only reached in women in POMS fatigue-inertia ( $9.6\pm 4.7$ ;  $5.9\pm 4.5$ ;  $8.1\pm 5.7$  for each group,  $p=0.006$ ) and confusion-bewilderment domains ( $3.8\pm 3.7$ ;  $1.3\pm 3.5$ ;  $3.1\pm 3.4$ ,  $p=0.004$ ), and QoL social relationships domain ( $13.0\pm 3.0$ ;  $14.6\pm 1.8$ ;  $13.9\pm 1.9$ ,  $p=0.031$ ). Inter-gender differences were observed in all groups, and were most pronounced during the first 5 years of the disease; men demonstrated significantly better scores across all domains with a notable exception of the POMS anger-hostility domain. The scores in the latter in men were higher throughout, reaching statistical significance in 5–10 years disease duration group ( $p=0.026$ ). An association of better QoL scores with higher HbA1c was observed and was significant in the social relationships domain in women ( $12.1\pm 3.2$ ;  $14.0\pm 2.1$ ;  $14.3\pm 1.9$ , respectively,  $p=0.024$ ). Gender differences were most marked in POMS vigor-activity and QoL environmental domains in the lower HbA1c range, and in QoL psychological domain in the higher HbA1c range. An association of increasing number of complications with lower QoL was significant in QoL psychological domain in women ( $12.3\pm 2.7$ ;  $12.1\pm 1.6$ ;  $13.7\pm 2.4$ ,  $p=0.016$ ) and in QoL physical domain in men ( $14.7\pm 1.9$ ;  $13.3\pm 2.5$ ;  $10.6\pm 0.4$ ,  $p=0.028$ ). The inter-gender differences were most marked in the complication-free group where women showed significantly worse results in POMS depression-dejection ( $p=0.010$ ), fatigue-inertia ( $p=0.008$ ), confusion-bewilderment ( $p=0.043$ ) domains, and in QoL physical ( $p<0.001$ ), psychological ( $p=0.001$ ) and environmental ( $p=0.035$ ) domains.

**Conclusion:** Quality of life and emotional status were best in patients with intermediate duration of T2DM; social aspects of QoL tended to improve



with an increasing HbA1c, possibly due to low cut-off values in HbA1c categories. Decreased QoL, as determined by scores in physical and psychological QoL domains, was significantly associated with increasing number of complications. The study found significant inter-gender differences in emotional status and quality of life scores; the differences in specific domains were most likely due to different strategies in coping with the disease.

## 966

### Differences in patient and clinician perspectives on type 2 diabetes mellitus management: the MOTIVATE global survey

P.E.H. Schwarz<sup>1</sup>, A.M. Felton<sup>2</sup>, M. Cobble<sup>3</sup>, S. Cavalcanti da Silva<sup>4</sup>, Y. Seino<sup>5</sup>, J. Wens<sup>6</sup>;

<sup>1</sup>Department of Medicine III, Technical University of Dresden, Germany, <sup>2</sup>FEND, London, UK, <sup>3</sup>Canyons Medical Center, Univ of Utah, Sandy, USA, <sup>4</sup>Endocrinology and metabolism, Santa casa de belo horizonte, Belo horizonte, Brazil, <sup>5</sup>Clinical Nutrition and Endocrinology, Kansai Electric Power Hospital, Osaka, Japan, <sup>6</sup>Primary and Interdisciplinary Care, University of Antwerp, Belgium.

**Background and aims:** Despite many therapeutic options and established treatment goals for patients with type 2 diabetes mellitus (T2DM), attaining combined goals for hyperglycaemia, weight reduction, blood pressure and dyslipidaemia remains difficult. The Measuring Opinions of Treatment Interventions In type 2 diAbetes mEllitus (MOTIVATE) global survey aims to improve understanding of the and differences in the perceptions of clinicians and patients regarding the treatment for T2DM.

**Materials and methods:** The MOTIVATE global survey assessed the perceptions that patients and primary care clinicians have on diabetes management beyond hyperglycaemia and evaluated weight management as a possible motivator of patient self-management. The MOTIVATE survey was an internet-based, 10-item questionnaire posed to patients with T2DM (n=2140) and clinicians (n=1406) randomly selected from 13 countries (US, Europe, Canada, China, Australia, Brazil, Mexico, Japan and South Africa) regarding attainment of various therapeutic goals and clinicians' understanding of their patients' anxiousness regarding these goals. Difference of  $\geq 3\%$  = statistically significant [95% CI], and  $\geq 10\%$  = clinically meaningful.

**Results:** Approximately 95% of patients and clinicians perceived the need for medications that address challenges beyond hyperglycaemia. Sixty percent of patients rated diabetic complications as one of their top three anxiety-provoking concerns while clinicians estimated that only 43% of their patients felt this way. Half of patients (52%) identified weight loss of  $>10\%$  as being meaningful while half of clinicians (51%) reported 1–5% weight loss as being meaningful; clinicians placed greater emphasis on durability of weight loss. Patients were more anxious about their blood pressure and lipid levels (19% and 18% reported as being very anxious, respectively) than clinicians perceived them to be (5% and 4%, respectively). Both patients and clinicians prefer a medication that offers modest weight loss (2.5%) even if accompanied by their least acceptable side effect. Patients and clinicians reported dizziness / fainting / hypoglycaemia as the least acceptable side effects.

**Conclusion:** The MOTIVATE global survey highlighted substantial disconnects between the perceptions of clinicians and patients regarding medication and therapeutic goals. These differences may impact the ability to attain desired T2DM treatment goals.

Supported by: AstraZeneca and Bristol-Myers Squibb

## 967

### GAPP2™: Global survey finds that a quarter of Japanese type 2 diabetes downplay insulin non-adherence to their healthcare professional

Y. Atsumi<sup>1</sup>, M. Brod<sup>2</sup>, A.H. Barnett<sup>3</sup>, M. Peyrot<sup>4</sup>;

<sup>1</sup>Saiseikai Central Hospital, Tokyo, Japan, <sup>2</sup>The Brod Group, Mill Valley, USA, <sup>3</sup>Heart of England NHS Foundation Trust and University of Birmingham, Birmingham, UK, <sup>4</sup>Loyola University, Baltimore, USA.

**Background and aims:** Type 2 diabetes (T2DM) rates are increasing in Japan due to a mix of genetic and environmental factors. Insulin analogue (IA) is widely initiated at diagnosis in Japan and a paternal physician-patient relationship remains common. To understand the impact of these issues, Japanese (JP) T2DM patients using IA and healthcare professionals (HCPs) were included in the GAPP2™ survey to assess the challenge of insulin irregularities and gain insights into differences between JP and other countries.

**Materials and methods:** An internet survey was carried out in US, Canada, Japan, UK, Germany and Denmark. HCPs involved in diabetes management and T2DM patients were identified from online research panels with over 6.5 million members. Data from 355 JP IA patients and 231 JP HCPs is compared with 2687 patients and 1422 HCPs from the five other survey countries (GP2).

**Results:** Although lower than other countries, JP patients reported irregularities of basal insulin (BI). In the last 30 days, 15% of JP patients reported missing doses of BI (mean 3.1 times) and 20% had missed 5+ doses (GP2, 22%; mean 3: 16% 5+ doses missed). JP HCPs said BI only patients told them they missed 1.5 doses per month (1.1 basal bolus patients (BB)), with just 11% admitting to 5+ missed BI doses (7% BB). GP2 HCPs reported higher numbers, mean 2.9 doses BI only (2.6 BB) and 21% missing 5+ doses (19% BB). In the same time period, 18% of JP patients reported mis-timing by  $> 2$  h (mean 5.4 times, 45% mis-timed 5+ doses), and 11% reducing a dose of BI (mean 3.8 times, 32% 5+ doses) compared to 25% (mean 4.1, 25% 5+ doses) and 15% (mean 4.2, 26% 5+ doses) for GP2 patients respectively. JP HCPs said that patients reported a significantly lower mean number of mis-timed and reduced BI doses (including those mis-timing/reducing 5+ doses) than GP2 HCPs. On the last occasion that JP patients had missed, mis-timed or reduced their BI dose, 15%, 15% and 69% had done so intentionally (13%, 17%, 77% of GP2 patients). For all patients the most common reasons for this were low blood sugar levels and hypoglycaemia risk reduction. 49% of JP patients (65% GP2) thought that missed BI doses would negatively impact their long-term health. However, more JP patients (55% vs 35% GP2) reported they would feel guilty if they missed a BI dose and JP patients were more likely to downplay the number of missed BI doses to their HCPs than GP2 respondents (22% vs 9%).

**Conclusion:** JP T2DM patients report substantial irregularities of BI dose which may lead to glycaemic control issues and long-term complications. Differences in reported rates compared to the GP2 IA population may be due to under-reporting, the impact of earlier insulin initiation or may reflect cultural differences in medication adherence. Cultural issues around reporting “failure” to HCPs require further study.

### Basal insulin injection non-adherence reported by T2DM patients using IA

	Missed BI dose		BI dose taken $> 2$ hrs earlier or $> 2$ hrs later than prescribed		Reduced BI dose	
	GP2	JP	GP2	JP	GP2	JP
Total (n)	2549	334	2407	334	2568	345
Ever	47%	38%*	54%	33%*	39%	30%*
More than 1 year ago	6%	6%	7%	2%*	6%	5%
Within last year	10%	10%	11%	6%*	9%	8%
Within last 90 days	9%	8%	12%	6%*	9%	6%*
Within last 30 days	22%	15%*	25%	18%*	15%	11%*
Mean number of times in last 30 days $\pm$ SE	3.0 $\pm$ 0.16	3.1 $\pm$ 0.64	4.1 $\pm$ 0.22	5.4 $\pm$ 0.71*	4.2 $\pm$ 0.26	3.8 $\pm$ 0.49
5+ times	16% (n=598)	20% (n=49)	25% (n=596)	45%* (n=60)	26% (n=37)	32% (n=380)

(\*statistically significant difference from GP2 at 95% level)

Supported by: The GAPP2 surveys were supported by a grant from Novo Nordisk

## 968

### Evaluation of the personality characteristics of type 1 patients in CSII therapy

A. Girelli<sup>1</sup>, D. Bruttomesso<sup>2</sup>, S. Ciaccio<sup>1</sup>, G. Grassi<sup>3</sup>, E. Cimino<sup>1</sup>, S. Costa<sup>2</sup>, A. Nicolucci<sup>4</sup>, R. Scotton<sup>2</sup>, U. Valentini<sup>1</sup>;

<sup>1</sup>Diabetes Care Unit, AO Spedali Civili, Brescia, <sup>2</sup>Diabetes Care Unit, AO University of Padova, <sup>3</sup>Division of Endocrinology, S. Giovanni Battista Hospital, Torino, <sup>4</sup>Mario Negri Sud, S. Maria Imbaro, Chieti, Italy.

**Background and aims:** The success of CSII (continuous subcutaneous insulin infusion) depends on the patient's proficiency and motivation. Few studies

have examined how personality influences the outcome of CSII. Aim of this multi-centric, observational, cross-sectional study was to describe the clinical and psychological characteristics of a large cohort of patients with type 1 diabetes treated with CSII and compare them with a group of patients treated with multiple daily insulin injections (MDI). We also studied the relationship between psychological characteristics and the effectiveness of therapy.

**Materials and methods:** 361 type 1 diabetic patients were sequentially recruited: 185 on CSII (61M / F124; disease duration  $20 \pm 10$  (SD) years; on CSII for  $5 \pm 4$  years) and 176 patients on MDI (M76 / F100; disease duration  $17 \pm 10$  years). Personality traits were assessed through validated questionnaires (BFQ-Big Five Questionnaire, Multidimensional Health Questionnaire-MHQ).

**Results:** Female patients were more numerous among patients on CSII. Considering metabolic control, glycated emoglobin levels were similar in the two groups (CSII= $7.8\% \pm 0.9$ , MDI= $7.7\% \pm 1.0$ ), but CHO counting was used more frequently ( $p=0.000$ ), and severe hypoglycemia was lower ( $p=0.032$ ) among patients on CSII. Patients in CSII showed significantly higher scores in the following domains: dynamism ( $p=0.050$ ), agreeableness ( $p=0.007$ ), friendliness ( $p=0.026$ ), openness to experience ( $p=0.011$ ), Health Efficacy ( $p=0.028$ ). Among CSII patients, those in better control ( $<7.5\%$ ) resulted have a higher score for conscientiousness ( $p=0.019$ ), Health-Efficacy ( $p=0.002$ ), Health Expectations-Optimism ( $p=0.034$ ), Health Esteem ( $p=0.0001$ ), Health Satisfaction ( $p=0.001$ ), Health Self-Schemata ( $p=0.036$ ) and Health Status ( $p=0.001$ ). Patients with poor control (HbA1c  $> 8.5\%$ ) showed higher scores for Health Anxiety ( $p=0.010$ ), Health Illness Self-Blame ( $p=0.024$ ), Health Monitoring ( $p=0.042$ ) and Health Depression ( $p=0.008$ ). When comparing CSII vs. MDI patients within the HbA1c  $<7.5\%$  range, CSII patients achieved average scores significantly higher on scales cooperativeness ( $p=0.013$ ), friendliness ( $p=0.024$ ), impulse control ( $p=0.033$ ), Health-Efficacy ( $p=0.009$ ), Health Consciousness ( $p=0.006$ ), Health Expectations-Optimism ( $p=0.044$ ), Illness Management ( $p=0.013$ ), Health Esteem ( $p=0.001$ ), Health Satisfaction ( $p=0.037$ ). Considering patients on CSII and MDI with HbA1c between 7.5–8.5%, those on CSII showed greater scores on scales openness to experience ( $p<0.009$ ) and Health-Efficacy ( $p<0.048$ ). There was no difference between CSII and MDI when HbA1c was above 8.5%.

**Conclusion:** Patients on CSII are more active and have a greater sense of self-efficacy than those in MDI. In patients on CSII, metabolic control is better in patients who are more conscientious, active, optimistic, have higher self-esteem and satisfaction with health status. This analysis confirms that psychological characteristics can significantly influence the success of CSII therapy and should be evaluated in the selection of patients.

**Results:** A 1 point lower score on the PF, GH, and PCS scales was associated with a 1.05–1.10 RR of mortality for the DM patients. The associations showed age dependence for these scales, with stronger associations in the younger age groups. For several scales (PF, RP, BP, GH, VT, SF, and RE), the associations with mortality also depended on score level, with stronger associations in the lower score ranges (i.e. among patients in worse health). A 1 point lower score on the PF, RP, BP, GH, SF, and PCS scales implied a 1.07–1.11 RR of being unable to work and a 1.05–1.06 RR of losing the ability to work. These associations were dependent on score level, with stronger associations in the higher score ranges. A 1 point lower score on the PF, RP, BP, GH, SF, and PCS scales was associated with a 1.03–1.04 RR of hospitalization. When baseline score and age were taken into account for each outcome, the results from the total sample did not differ significantly from the DM patients' results. Gender, education, marital status, and comorbidity score had only a minor impact on the strength of any of the associations.

**Conclusion:** Statistical associations with outcomes such as mortality, work ability, and risk of hospitalization can be used as benchmarks to interpret the magnitude of scale score differences for PROs. For a diabetes population, the results indicate up to 10% excess risk of mortality and inability to work for a 1 point lower score on selected SF-36 scales.

*Supported by: Novo Nordisk A/S*

## 969

### Benchmarks for interpretation of score differences on the SF-36 health survey for diabetes patients

J.B. Bjorner<sup>1,2</sup>, M.L. Wolden<sup>3</sup>, J. Gundgaard<sup>3</sup>, K. Miller<sup>1</sup>;

<sup>1</sup>QualityMetric, Lincoln, USA, <sup>2</sup>Department of Public Health, University of Copenhagen, <sup>3</sup>Novo Nordisk, Copenhagen, Denmark.

**Background and aims:** Patient-reported outcomes (PROs) are widely used in clinical research. However, results on score differences may be hard to interpret if clinicians are unfamiliar with the assessment tools and lack benchmarks for interpretation of results. This study aims to establish an interpretation of score differences on the SF-36 Health Survey by linking score differences to clinical and social outcomes, and to apply this interpretation model to a diabetes mellitus (DM) population. Further, it tests whether the interpretation depends on score level and patient background characteristics.

**Materials and methods:** Using survival and logistic regression models, data from the following 3 large US cohort studies was reanalysed: the Medical Outcomes Study (N=3,445; 541 DM patients), the Medicare Health Outcomes Study (N=78,183; 16,388 DM patients), and the QualityMetric 2009 Norming Study (N=4,040; 580 DM patients). Outcome variables were mortality, hospitalization within 6 months after baseline, inability to work at baseline, and loss of ability to work within 6 months after baseline. The independent variables included scale score, age, gender, education, marital status, and number of comorbidities. Separate analyses tested scales concerning Physical Function (PF), Role Limitation due to Physical Health (RP), Bodily Pain (BP), General Health (GH), Vitality (VT), Social Function (SF), Role Limitation due to Emotional Problems (RE), Mental Health (MH), the Physical Component Summary (PCS), and the Mental Component Summary (MCS). Regression models from the total samples were applied to the DM patients and the estimated relative risks (RR) were compared to the results of separate analyses for the DM patients.

## PS 080 Psychological aspects: stress and depression

970

### Depressive symptoms in type 1 diabetic children with poor and excellent metabolic control

B.M. Zdunczyk<sup>1</sup>, J. Sendela<sup>2</sup>, H. Trippenbach-Dulska<sup>3</sup>, M. Lipka<sup>2</sup>, M. Procter-Czaplinska<sup>2</sup>, L. Groele<sup>2</sup>, A. Szybowska<sup>3</sup>;

<sup>1</sup>Clinical Child and Adolescent, Clinical Pediatric Hospital, <sup>2</sup>The Department of Pediatrics, Clinical Pediatric Hospital, <sup>3</sup>Department of Pediatrics, Medical University of Warsaw, Poland.

**Background and aims:** Many studies show there is a higher incidence of diabetes among patients with depression and depression is more often diagnosed in patients with diabetes than in healthy population. Depression is considered a major complication negatively affecting metabolic control in patients with diabetes. The aim of this study was to assess the prevalence of depression and examine its effect on metabolic control and quality of life in children and adolescents with diabetes type 1.

**Materials and methods:** 477 children took part in this study: 252 girls and 225 boys with diabetes duration > 1 year. Mean age: 13.1 years <7–17, SD 2.7>. Mean diabetes duration: 5 SD 3.5 years, mean HbA1c 7.5% <5–13.9, SD 1.5>. During the routine visit in the outpatient clinic all children and adolescents with diabetes type 1 age 7 and above were asked to fill in Polish version of Children's Depression Inventory by M. Kovac, a self-report questionnaire consisting of 27 items. Patients from age 11 and above were additionally asked to answer questions in 58-item Quality of Life Questionnaire, based on the DCCT Diabetes Quality of Life Measure. At the same time other data was collected: sex, age, diabetes duration, HbA1c, BMI, daily insulin dose.

**Results:** 17% (81/477) participants scored  $\geq 13$ , indicating elevated depressive symptoms. We found significant relationship between scores on CDI and HbA1c ( $r=0.16$ ,  $p=0.0002$ ). 181/477 (38%) children achieved mean HbA1c  $\leq 7\%$ , in this group 13% (24) subjects had CDI  $\geq 13$ . There was no difference between the frequency of depressive symptoms in children with HbA1c  $\leq 7\%$  ( $p=0.102$ ). We also didn't find the difference between participants with CDI  $\geq 13$  and CDI < 13 in HbA1c ( $p=0.24$ ). There was an extremely significant correlation between scores on the CDI and quality of life ( $r=0.64$ ,  $p<0.0001$ ), age of participants ( $r=0.24$ ,  $p<0.0001$ ) and BMI ( $r=0.22$ ,  $p<0.0001$ ). We found the difference between children with CDI  $\geq 13$  and CDI < 13 in BMI ( $p=0.0293$ ). There was also a significant correlation between scores in the CDI and insulin daily dose ( $r=0.10$ ,  $p=0.0229$ ). In the group with HbA1c  $\leq 7\%$  children with CDI  $\geq 13$  and CDI < 13 didn't differ in BMI or daily insulin dose. 9.8% (47) participants reported suicidal thoughts, which have a significant impact on CDI scores ( $P<0.0001$ ) and quality of life ( $P<0.0001$ ) but not on HbA1c ( $P=0.1106$ ). There was no difference between girls and boys on the CDI scores.

**Conclusion:** 17% participants show elevated depressive symptoms as assessed with Children's Depression Inventory. Children and adolescents with higher scores on the CDI are older, have worse quality of life, higher BMI, daily insulin dose as well as achieve worse metabolic control. Still they achieve similar level of HbA1c as their peers with lower CDI scores. Depressive symptoms were noted in 13% children with excellent control of diabetes (HbA1c  $\leq 7\%$ ). It is necessary to pay attention to emotional wellbeing of children and adolescents with diabetes type 1 regardless of HbA1c. An intervention program aimed at prevention of emotional problems in youths with diabetes should be developed. Depressive symptoms may be no more a major factor in adherence to treatment but more studies are required.

971

### The assessment of factors determining fatigue in subjects with long history of type 1 diabetes

A. Duda-Sobczak, D. Zozulinska-Ziolkiewicz, B. Wierusz-Wysocka; Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poland.

**Background and aims:** Type 1 diabetes is a chronic disease requiring continuous insulin therapy. Adjusting the rules of treatment to the existing lifestyle can be a source of emotional distress and the feeling of fatigue. The aim of this study was to assess the factors determining the fatigue in subjects with long history of type 1 diabetes.

**Materials and methods:** 213 subjects (116 women), aged <60 years (26.6

$\pm 6.0$ ), diabetes duration >20 years ( $42.2 \pm 10.5$ ), HbA1c  $8.2 \pm 1.4\%$  ( $66 \pm 7.0$  mmol/mol) were included. Subjects diagnosed with chronic complications causing disability (blindness, end stage renal disease, painful peripheral neuropathy, limb amputation) were excluded. Patients fulfilled questionnaire comprising the question: Do you feel fatigue due to living with diabetes? Additionally, Problem Areas in Diabetes Questionnaire (PAID), Beck Depression Inventory (BDI), NEO-FFI (Neuroticism-Extraversion-Openness Five Factor Inventory) were completed. Socioeconomic status and parameters of metabolic control were assessed.

**Results:** 57% of subjects (61% of women, 51% of men) declared fatigue due to diabetes. Economically active people were less tired of disease ( $p=0.001$ ), subjects with higher education level less frequently declared fatigue ( $p=0.03$ ). The fear of hypoglycemia was more frequent in group declaring fatigue due to diabetes ( $p=0.001$ ).

**Conclusion:** Fatigue in type 1 diabetes is determined by comorbid depressive disorder, difficulty coping with diabetes, neurotic personality and some socioeconomic factors.

### Characteristics of group declaring and denying fatigue due to diabetes

	Declared fatigue due to living with diabetes		P
	yes	no	
HbA1C [%] (mmol/mol)	8,1 $\pm$ 1,2 (65 $\pm$ 6,0)	8,2 $\pm$ 1,7 (66 $\pm$ 8,5)	0,899
BDI [score]	12,7 $\pm$ 8,8	6,9 $\pm$ 5,9	0,000001
PAID [score]	37,0 $\pm$ 21,0	19,7 $\pm$ 13,5	0,000001
Personality factors			
Conscientiousness [score]	33,0 $\pm$ 6,6	34,0 $\pm$ 5,7	0,211
Openness to Experience [score]	25,0 $\pm$ 5,5	25,5 $\pm$ 5,5	0,262
Extraversion [score]	26,1 $\pm$ 5,8	28,7 $\pm$ 6,2	0,0004
Neuroticism [score]	23,9 $\pm$ 8,4	17,7 $\pm$ 7,1	0,000001
Agreeableness [score]	31,0 $\pm$ 5,5	31,5 $\pm$ 4,5	0,540

972

### The triple P study: psychopathology in pump patients

P.S. Grant;

Diabetes & Endocrinology, Tunbridge Wells Hospital, Pembury, UK.

**Background and aims:** CSII is generally successful for patients with type 1 diabetes in improving glycaemic control, alleviating the burden of hypoglycaemia and improving quality of life. There is however, a cohort of patients who fail to thrive on pump therapy and psychological factors or 'brittleness' have been posited as a cause for this. We aimed to assess the extent and spectrum of psychological illness in a population of pump patients.

**Materials and methods:** We analysed the patient data and records of 350 patients with type 1 diabetes who formed the insulin pump patient population from a large teaching hospital and compared them with an age and sex matched reference population of patients with type 1 diabetes. We quantified the prevalence of mental health problems both before and after the initiation of pump therapy and looked to see whether there was a difference in terms of changes in glycaemic control.

**Results:** In this analysis, it appears that mental health problems amongst patients selected for CSII occur significantly more frequently than in a matched population of type 1 diabetics. Of those with psycho-pathology, they have a tendency to do less well in terms of reduction in glycaemic control as indicated by changes in HbA1c, however hypoglycaemia incidence did not appear to differ significantly either.

**Conclusion:** The incidence and prevalence of mental health problems in individuals with diabetes is greater than that of the general population. In patients who go on to be selected to have insulin pump therapy, the incidence is again greater. We have shown that in those with psychological illness they tend to do less well in terms of improving their overall diabetes control, with some patients even doing worse than before. These results show that CSII may not be a suitable route of therapy for all of those who would fulfill the traditional criteria and suggest that psychological assessment, therapy and intervention may be an altogether more appropriate course of action in supporting their diabetes self management. The wider implications are that all patients with diabetes should be regularly assessed for psychological problems and that there needs to be greater psychology / psychiatric support available to diabetes clinics.



## 973

**The diabetes education program affects the depression score of type 2 diabetic patients**

T. Niya<sup>1</sup>, S. Furukawa<sup>1,2</sup>, Y. Tamagawa<sup>1</sup>, S. Shojima<sup>1</sup>, K. Manabe<sup>1</sup>, A. Ogawa<sup>1</sup>, E. Kawamoto<sup>1</sup>, T. Ueda<sup>3</sup>, H. Tokunaga<sup>3</sup>, O. Ebisui<sup>3</sup>;

<sup>1</sup>Department of Internal Medicine (Diabetes & Endocrinology), Matsuyama Shimon Hospital, <sup>2</sup>Department of Gastroenterology and Metabolism, Ehime University Graduate School of Medicine, Toon,

<sup>3</sup>Department of Diabetes & Endocrinology, Ehime Prefectural Central Hospital, Matsuyama, Japan.

**Background and aims:** Recently, many reports have shown that depressive symptoms in type 2 diabetic patients are linked with hyperglycaemia and many other diabetic complications. Treatment of diabetes may affect the depressive symptoms in patients with type 2 diabetes. In addition, when the patients understand their prognosis with regard to diabetic complications, they may feel depressed. Thus, while performing the diabetes education program for type 2 diabetic patients, it is necessary to be careful about depressive symptoms. The aim of this study was to investigate the effect of a diabetes education program on depressive symptoms.

**Materials and methods:** We enrolled 528 patients with type 2 diabetes who were admitted to our hospital to participate in the diabetes education program [age:  $60.8 \pm 11.1$  years, M/F: 330/198, HbA1c level:  $9.7\% \pm 1.8\%$ ]. We evaluated their depressive symptoms using the Zung Self-Rating Depression Scale (SDS) before and after the diabetes education program. In the program, we managed to achieve glycaemic control; in addition, we detected the presence of diabetic complications and performed self-management education for 2 weeks during hospitalization. We investigated the changes in the SDS score and the scores of 3 sub-scales (pervasive effects, physiological equivalents, and psychological equivalents) before and after the diabetes education program. Statistical evaluation was performed using a Wilcoxon signed-rank test. The level of significance was set at  $P < 0.05$ .

**Results:** Before we performed the diabetes education program, the number of diabetic patients in a non-depressive state was only 232 (43.9%). There were 167 (31.6%) and 129 (24.4%) diabetic patients in mild and moderate/severe depressive state, respectively. The SDS score decreased significantly in all patients in a depressive state ( $40.6 \pm 8.9$  to  $39.7 \pm 9.0$ ,  $P = 0.0025$ ); it decreased from  $43.4 \pm 2.3$  to  $41.3 \pm 6.5$  ( $P < 0.0001$ ) in patients in a mild depressive state and from  $51.9 \pm 3.6$  to  $47.8 \pm 6.9$  ( $P < 0.0001$ ) in those in a moderate/severe depressive state. On the other hand, the SDS score of patients in a non-depressive state increased significantly ( $32.2 \pm 4.5$  to  $34.1 \pm 7.5$ ,  $P = 0.0007$ ). In the sub-scales, the score of psychological equivalents decreased significantly in all patients ( $21.1 \pm 6.4$  to  $20.5 \pm 6.5$ ,  $P = 0.0003$ ). However, the score of pervasive effects and physiological equivalents did not change significantly in all patients. There was a significant increase in the score of physiological equivalents ( $14.6 \pm 2.7$  to  $15.1 \pm 2.8$ ,  $P = 0.0043$ ) and psychological equivalents ( $15.3 \pm 3.5$  to  $16.6 \pm 5.6$ ,  $P = 0.0098$ ) in patients in a non-depressive state. Nevertheless, the score of physiological equivalents and psychological equivalents decreased significantly in patients in a mild depressive state ( $17.4 \pm 2.8$  to  $16.8 \pm 2.9$ ,  $P = 0.0147$  and  $23.3 \pm 3.4$  to  $21.9 \pm 5.1$ ,  $P < 0.0001$ , respectively) and moderate/severe depressive state ( $20.1 \pm 3.0$  to  $19.0 \pm 3.6$ ,  $P = 0.0036$  and  $28.5 \pm 3.5$  to  $25.7 \pm 5.1$ ,  $P < 0.0001$ , respectively).

**Conclusion:** These results suggest that a diabetes education program may affect the physiological equivalents and psychological equivalents of the depressive score in type 2 diabetic patients.

## 974

**Poor adherence to therapy of depression in diabetes patients with insufficient metabolic control: results of the Diabetes Depression Study (DAD study)**

F. Petrak<sup>1</sup>, S. Herpertz<sup>1</sup>, C. Albus<sup>2</sup>, N. Hermanns<sup>3</sup>, K. Kronfeld<sup>4</sup>, B. Kulzer<sup>5</sup>, J. Kruse<sup>6</sup>, C. Ruckes<sup>4</sup>, M.J. Müller<sup>2</sup>;

<sup>1</sup>Clinic of Psychosomatic Medicine and Psychotherapy, LWL-University Clinic Bochum, <sup>2</sup>Klinik und Poliklinik für Psychosomatik und Psychotherapie, Universitätsklinikum, Köln, <sup>3</sup>Forschungsinstitut Diabetes-Akademie (FIDAM), Bad Mergentheim, <sup>4</sup>Interdisziplinäres Zentrum Klinische Studien (IZKS), Universitätsmedizin der Universität, Mainz, <sup>5</sup>Klinik für Psychosomatik und Psychotherapie, Universitätsklinikum, Gießen, <sup>6</sup>Vitos Klinikum, Marburg und Gießen-Marburg, Germany.

**Background and aims:** It is well established that depression impairs adherence to diabetes treatment. But, it is unclear if this is also the case for depres-

sion treatment. Therefore, we analysed adherence to therapy of depression in poorly controlled diabetes patients within a randomised controlled multicentre trial.

**Materials and methods:** Diabetes patients (type 1 or 2) with depression (Structured Clinical Interview for DSM-IV) were randomised to 50-200mg Sertraline (SER) or 10 sessions of a diabetes specific cognitive behavioural group therapy (CBT). Response regarding depression was assessed after 12 weeks (Hamilton Depression Rating Scale, HAMD: Baseline - 50% or  $\leq 7$ ). Adherence to treatment was defined by measuring the received therapy 'dose'; number of attended sessions were counted for the CBT group (0=nonadherent; 1-7=partially nonadherent; 8-10=adherent). For the SER group analyses of sertraline and N-desmethylsertraline in blood serum were performed after 8 and 12 weeks of treatment to measure adherence to treatment and serum target ranges were defined. Patients with SER concentrations twice within the target range were considered as adherent, once within the target range as partially nonadherent, and never within the target range as nonadherent. Group differences (adherent vs partially nonadherent or nonadherent) were analysed regarding patients characteristics and response to therapy using t-tests or Chi<sup>2</sup>-Tests.

**Results:** Within the CBT group (N=126) 54% (68/126) of patients were considered as adherent, 26.2% (33/126) as partially nonadherent and 19.8% (25/126) as nonadherent. For the SER group (N=120) 38.3% of patients were adherent (46/120), 28.3% (34/120) partially nonadherent, and 33.3% (40/120) nonadherent. Non-adherent patients were treated more often with insulin pumps (N=28 vs 13;  $p < 0.05$ ), they had a higher HbA<sub>1c</sub> ( $9.4 \pm 1.6$  vs.  $9.0 \pm 1.3$ ;  $p < 0.05$ ) and they suffered more often from very severe depression (HAMD $>25$ , N=16 vs. 3,  $p < 0.05$ ) compared to adherent patients. Significantly higher response rates were observed after 12 weeks of SER treatment for adherent patients compared to the partially adherent or nonadherent group ( $p < 0.05$ ), a difference that could not be observed within the CBT group.

**Conclusion:** A majority of patients did not adhere to their treatment. Only 54% of the CBT patients participated sufficiently in the therapy sessions and the vast majority of patients treated with sertraline did not take their medication as intended. Nonadherence predicted nonresponse for the sertraline treatment but not for the CBT.

Clinical Trial Registration Number: ISRCTN 89333241

Supported by: Competence Network for Diabetes mellitus funded by BMBF (FKZ: 01KG0505)

## 975

**Temporal changes in the prevalence of depression and diabetes mellitus in a rural population of Bangladesh: from 2004 to 2009**

K. Natasha, B. Bhowmik, S. Asghar, A. Hussain;

Community Medicine, Institute of health and society, Faculty of Medicine, Oslo, Norway.

**Background and aim:** Unfortunately having least data on depression among rural diabetic people in Bangladesh we had been working in this field for last 2 decades. Referred to a previous study in that particular area, whether the situation is same or changed, we designed the study to freshly investigate the prevalence of depressive symptoms in individuals with and without diabetes in 2009.

**Method:** 952 and 2293 subjects aged  $\geq 20$  years in 2004 and 2009 respectively were collected from 10 villages of a rural community. Type 2 Diabetic cases (DM) were defined using the diagnostic criteria by World Health Organization. Simplified Montgomery and Asberg (MADRS) scale ( $\geq 20$  indicated depression) was followed for depression scoring. Risk indicators for depression in relation to socio-economic state, education, marital status and glucose abnormality, anthropometric measurements and gross clinical conditions were also assessed.

**Results:** Table: Selected socio demographical, economical and clinical data of the study population

	Healthy		DM only	Depression Only	Both			
	2004	2009	2004	2009	2004	2009	2004	2009
N (%)	648 (68.07%)	2038 (88.87%)	27 (2.84%)	148 (6.45%)	120 (12.61%)	74 (3.23%)	157 (16.49%)	33 (1.44%)
Age (Mean ±SD)	38.5 ± 12.3	41.42 ± 13.80	42.0 ± 9.0	45.01 ± 2.99	41.2 ± 14.8	45.59 ± 15.46	44.5 ± 8.8	43.09 ± 12.12
Male (%)	42.60	37.54	70.40	22.30	37.50	41.89	45.8	39.39
Female (%)	57.40	62.46	29.60	77.70	62.50	58.11	54.2	60.61
Monthly income (Taka) (Mean ±SD)	6658.00 ± 6648.00	7763.33 ± 5755.41	9463.00 ± 7367.00	8672.30 ± 6713.70	5917.00 ± 5747.00	8135.14 ± 5408.19	8685.00 ± 6194.00	10393.94 ± 6791.26
Physical activity (high)	72.40	73.99	81.5	65.54	74.20	62.16	68.8	66.67
BMI (Mean ±SD)	21.6 ± 4.0	22.46 ± 3.717	23.5 ± 3.2	24.77 ± 4.920	20.6 ± 3.2	21.76 ± 4.355	23.8 ± 3.8	25.60 ± 4.862
Systolic Blood Pressure (Mean ±SD)	117 ± 18	114.90 ± 16.075	123 ± 13	121.86 ± 17.036	119 ± 21	115.47 ± 20.139	123 ± 19	117.73 ± 17.098
WHR (Mean±SD)	0.88 ± 0.13	0.87 ± 0.06	0.93 ± 0.05	0.91 ± .06	0.86 ± 0.06	0.86 ± 0.07	0.93 ± 0.19	0.93 ± 0.06

\*\*1USD=68Tk(2004), 69Tk(2009)

Over all prevalence of Depression in 2004 and 2009 were 29% and 4.67% (95% CI) respectively though the mean age between the two studies were different (39 in 2004 and 41 in 2009). The depressive symptoms in subject with diabetes and without diabetes were 29.7% and 14.1% in 2004 and 18.2% and 2.3% in 2009.

**Discussion and conclusion:** The prevalence of depression in the later study was low specially in females, compared to the previous one, and more similar with the studies from National Institute of Mental Health, Bangladesh (4.6%), neighbour countries like Pakistan (5.8%) and India (7.4%). The systolic blood pressure declined also. The study supported the trend of diabetes which was upward (2.8% in 2004 and 6.45% in 2009). Improvements in the mean BMI score (according to WHO cut off point for Asian people) from 20.2±3.0 to 22.63 ± 3.9 and the mean financial status (USD 112.80 ± 83.62) was predicted to be the cause. It also could be predicted that less earning capacity and lack of socio technical facilities are the main cause of depression among people like ours. Our study area had expansions in their small (36.3%) and medium (46.2%) trade, electric supply (3.5%), sanitation (4.1%), health services and road and transport facilities within those 5 years the disease scenario changed. We can conclude with a stance that depression in rural developing area is based on low economic status. When the life standard increases the chance of NCD increases but depressive symptoms turn down.

Supported by: University of Oslo, Norway

## 976

### Validation of the Danish version of diabetes distress scale

L.E. Joensen<sup>1</sup>, I. Tapager<sup>2</sup>, I. Wilaing<sup>1</sup>

<sup>1</sup>Steno Health Promotion Center, Steno Diabetes Center, Gentofte, Denmark,

<sup>2</sup>Incentive Partners, Holte, Denmark.

**Background and aims:** Psychosocial problems are highly prevalent among diabetes patients. Different measures aim to assess the extent and the burden of psychosocial problems among persons with diabetes. The purpose of this study was to examine the internal consistency and validity of a Danish translation of the Diabetes Distress Scale (DDS) among adults with type 1 diabetes.

**Materials and methods:** Data was collected with a large cross-sectional survey of adults with Type 1 diabetes (N=2419, response rate 67%) in 2011. Validated scales and questions measured diabetes distress, diabetes empowerment (DES-SF), self-management behaviours, self-rated health (SF12) and quality of life (WHOQoL). An electronic patient record provided information about glycemic control (HbA1c). The Diabetes Distress Scale (DDS) consists of 17 items describing possible diabetes-related problems. The total score is derived as the sum of the 17 item scores divided by 17. The DDS is originally subdivided into four domains of diabetes related distress: emotional burden (EB), physician-related distress (PD), regimen-related distress

(RD) and diabetes-related interpersonal distress (ID). The original DDS was translated into Danish using an academic forward-backward translation procedure. Data was analyzed by computing Cronbach's alpha, factor analyses, t-test and pairwise Pearson correlations.

**Results:** Relatively few of the respondents (5.1%) left items in the scale unanswered. Overall, an acceptable number of respondents (6.3%) had the lowest possible score whereas none reached the maximum diabetes distress score. Exploratory factor analyses of DDS suggested that a 3 or 4 factor division into subscales was appropriate. If 4 factors were modeled, the division into factors corresponded almost precisely to the four domains described in the development of DDS. Only one item departed from the expected grouping and was highly correlated with the EB subscale as well as the RD subscale. The total scale had a Cronbach's  $\alpha$  of 0.92. The mean DDS-score was slightly higher for women than for men (2.0 vs. 1.8,  $p < 0.001$ ). Younger adults reported more diabetes related distress than older adults and adults with shorter diabetes duration reported more diabetes distress than adults with longer diabetes duration. Higher distress scores was correlated with low diabetes empowerment, low quality of life, unhealthy diet, not being physically active, poor glycemic control and low scores on the SF12 mental and physical component score. Analyses of HbA1c and DDS showed the highest correlation for regimen-related distress. The correlation with the SF12 physical component score was smaller than the correlation with the mental component.

**Conclusion:** Our results provide support for the use of this Danish adaptation of the 17 item Diabetes Distress Scale among adults with type 1 diabetes for screening for psychosocial distress. The scale has high internal consistency (Cronbach's  $\alpha$ 's) and the correlations with external measures confirm our hypotheses on the covariations of the underlying concepts. This supports the validity of the scale. The cross sectional nature of the study does not allow us to take into account possible day-to-day variation, test-retest reliability or sensitivity to changes over time. However, it is a testament to the reliability of the scale that the overall results of the analyses correspond well to previous investigations of the original version of the scale.

## 977

### Reliability and validity of the Chinese version of diabetes distress screening scale

M. Li, Y. Mo, L. Ji;

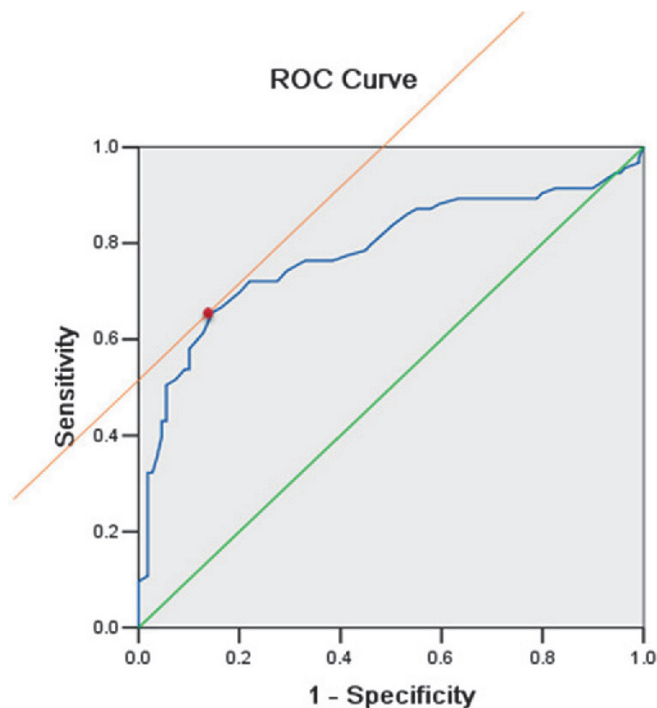
Peking University, Beijing, China.

**Background and aims:** Diabetes related distress is a common condition of patients with diabetes, which includes high levels of negative affect and associated with poor disease management. Diabetes Distress Screening Scale is a specific instrument that measures the diabetes related distress. The scale has been used widely in research and clinical practice. However, no published data are currently available on the instrument for the Chinese population. The aim of this study was to examine the psychometric properties of a Chinese version of the DDS scale (DDS-C).

**Materials and methods:** The reliability and validity of the DDS-C were evaluated in 202 outpatients with type 2 diabetes. Cronbach's  $\alpha$  coefficient, factor analysis, spearman's correlation and ROC curve facilitated the psychometric evaluation. The Self-rating Depression Scale (SDS) and Self-rating Anxiety Scale (SAS) scale were adopted to evaluate the criterion-related validity. One week test-retest reliability was measured.

**Results:** Exploratory factor analysis identified 4 sub-dimensions: emotional burden (EB), physician-related distress (PD), diabetes-related interpersonal and regimen distress (IRD), and medical resources-related distress (MRD). The last sub-dimension was different from the original scale which may reflect the cultural difference. The criterion-related validity of DDS-C between both SDS and SAS were acceptable ( $r_{\text{SDS}} = 0.625$ ,  $r_{\text{SAS}} = 0.633$ ,  $p < 0.01$ ). The internal consistency was good (Cronbach's  $\alpha = 0.892$ , Guttman split-half reliability = 0.895). The one week test-retest reliability was satisfactory ( $r_s = 0.9044$ ,  $p < 0.01$ ). The boundary value indicating depression emotion estimated by ROC curve was 47.5 with sensitivity of 0.656 and specificity of 0.85 while the boundary value indicating anxious emotion was 47.5 as well with sensitivity of 0.73 and specificity of 0.82.

**Conclusion:** This study suggested that the DDS-C has a consistent, generalizable factor structure with good internal reliability and validity, which could be used as a valuable instrument to assess diabetes-related distress in Chinese patients.



found between the diabetics on oral diabetic medication versus the insulin treated.

**Conclusion:** Diabetes in IHD seems specifically associated with increased depression score and lower physical quality of life. Splitting the diabetics into 2 groups of either high-high or low-low PPS and CSS seemed to gather all the different stress Q's, and the combination of the 2 might be usable for future research on the chronic stress burden in diabetes and IHD.

*Clinical Trial Registration Number: NCT01513824*

*Supported by: Johan Schrøders Familie og Erhvervsfond*

## 978

### Chronic stress as evaluated by pressure pain sensitivity and questionnaires in patients with ischaemic heart disease, with and without diabetes

N. Bergmann<sup>1</sup>, S. Ballegaard<sup>2</sup>, L. Andersen<sup>3</sup>, F. Gyntelberg<sup>4</sup>, C. Kistorp<sup>1</sup>, P. Holmager<sup>1</sup>, J. Faber<sup>1</sup>;

<sup>1</sup>Center of Endocrinology and Metabolism, Herlev University Hospital, Copenhagen, <sup>2</sup>Ull Care A/S, Hellerup, <sup>3</sup>Department of Cardiology P, Gentofte University Hospital, <sup>4</sup>The National Research Center for the Working Environment, Copenhagen, Denmark.

**Background and aims:** Diabetes and ischemic heart disease (IHD) are associated with chronic stress, which is a prognostic risk factor of mortality in IHD. Known questionnaires (Q's) of chronic stress covers typically only parts of the global stress concept. A newly developed hand-held device measures hyperalgesia, which seems to be stress-induced, on the sternum as Pressure Pain Sensitivity (PPS). PPS measurement is easy to perform, and might be used as a measure of chronic stress. The aim of the present study was to investigate chronic stress and the distribution of the different elements of the chronic stress-concept in diabetic patients with established and stable IHD, and to validate PPS and a newly developed 59 items stress score (CSS), both regarded as potential measures of the global concept of stress.

**Materials and methods:** A cross-sectional study of 361 patients with established and stable IHD of whom 47 (13%) had diabetes. Chronic stress was evaluated by PPS and by the following Q's: CSS, Quality of Life (SF-36), depression (MDI) and recent well-being (WHO-5). PPS was measured on the most sensitive point on sternum in the area of IC-4 to 5

**Results:** IHD patients with diabetes had a higher MDI score ( $r = 0.12$ ,  $p = 0.019$ ), lower SF-36 physical component summary score ( $r = -0.17$ ,  $p = 0.001$ ) and a lower score of several sub-measurements of the SF-36 as compared to non-diabetic IHD patients. No differences were found between diabetics and non-diabetics with regard to PPS or the other Q's. However, when comparing the most stressed diabetics, i.e. those with the highest tertiles of PPS and of CSS (high-high group) vs. those with the lowest tertiles of PPS and CSS (low-low group), the high-high group had increased MDI and reduced WHO-5 score and SF-36 mental component summary score ( $p = 0.048$ ,  $p = 0.047$  and  $p = 0.031$ , respectively). Compared to the non-diabetic IHD patients, the diabetics had higher angina score ( $r = 0.16$ ,  $p = 0.002$ ) but similar NYHA-score. CSS correlated to both angina- and NYHA score in both the diabetics and non-diabetics (all  $p < 0.001$ ) No differences in stress-measurements were



## PS 081 Clinical diabetes care

979

### Impact of bariatric surgery on life expectancy in adults with diabetes

P.J. O'Connor<sup>1</sup>, D. Schauer<sup>2</sup>, D. Arterburn<sup>3</sup>, E. Livingston<sup>4</sup>, K. Coleman<sup>5</sup>, S. Sidney<sup>6</sup>, D. Fischer<sup>7</sup>, M. Eckman<sup>8</sup>;

<sup>1</sup>HealthPartners Research Foundation, Minneapolis, <sup>2</sup>Division of General Internal Medicine, University of Cincinnati Medical Center, <sup>3</sup>Center for Health Studies, Group Health, Seattle, <sup>4</sup>Dept. of Gastrointestinal and Endocrine Surgery, University of Texas, Dallas, <sup>5</sup>Southern California Permanente Medical Group, Pasadena, <sup>6</sup>Kaiser Permanente Northern California, Oakland, <sup>7</sup>University of Cincinnati Medical Center, <sup>8</sup>Division of General Surgery, University of Cincinnati Medical Center, USA.

**Background and aims:** To determine the impact of gastric bypass on life expectancy for severely obese patients with diabetes.

**Materials and methods:** We developed a decision-analytic Markov model to evaluate two strategies for severely obese patients with diabetes: gastric bypass versus nonsurgical treatment. The efficacy of surgery was determined from a retrospective cohort of 165,000 severely obese diabetic patients (4500 had gastric bypass) from 4 HMO Research Network sites using fully adjusted Cox proportional hazards models. Logistic regression models calculated in-hospital mortality for surgery using data from the Nationwide Inpatient Sample (NIS). The decision model was calibrated using data from the National Health Interview Survey that is linked to the National Death Index. The model was constructed using Decision Maker<sup>®</sup>, which estimated changes in life expectancy.

**Results:** Our base case, a 40 year-old female with a BMI of 45, no hypertension, no coronary artery disease and no congestive heart failure, gained an additional 7.1 years of life expectancy with gastric bypass (43.1 years with surgery vs. 36.0 without). Surgery was no longer favored in our base case when 30-day surgical mortality exceeded 16% (baseline risk was 0.2%). Sensitivity analyses revealed that the gain in life expectancy decreases with increasing BMI, until a BMI of 62 is reached; at this point, nonsurgical treatment is associated with greater life expectancy than gastric bypass. Similar results for both men and women in all age groups were seen.

**Conclusion:** For most severely obese diabetic patients, gastric bypass surgery increases life expectancy; however, gastric bypass decreases life expectancy for those with a BMI over 62. Patients with high BMI may have other benefits from surgery, such as better quality of life and reduced burden of comorbid disease.

Supported by: AHRQ (HHSA290-2005-0033) and NIDDK (DK092924)

980

### Adherence to GLP-1 agonist therapy in US managed care

E. Chou<sup>1</sup>, M. Germe<sup>2</sup>, S. Schwartz<sup>3</sup>;

<sup>1</sup>R&D, Sanofi, Bridgewater, USA, <sup>2</sup>R&D, Sanofi, Paris, France, <sup>3</sup>Main Line Health System, Wynnewood, USA.

**Background and aims:** Non-adherence is a major obstacle for outcomes improvement in diabetes care. This study evaluated medication adherence with GLP-1 Agonist (GLP1) therapy.

**Materials and methods:** Patients who initiated a GLP-1 therapy during Apr 2005 to Dec 2010 with 1 year follow-up were identified from the Impact<sup>™</sup> Database. Adherence to GLP1 was approximated by medication possession ratio (MPR) as the ratio of days a patient had GLP1 drug in possession relative to the calendar days in the year after the drug initiation. Yearly adherence was defined as MPR ≥80%.

**Results:** Mean age of exenatide (EXEN: n=52,898) and liraglutide (LIRA: n=1,587) initiators was 53 years. Yearly adherence rate for EXEN decreased from 34% in 2005 to 20% in 2010, and was 31% in 2010 for LIRA. In A1C responders (<7%), adherence rate to both GLP-1 therapies was higher than in non-responders (OR=2.21; 1.98-2.46). The yearly rate of medical claims for nausea or vomiting was 4.5-5.2% for EXEN and 6.5% in 2010 for LIRA. Specifically for EXEN, nausea or vomiting were related with non-adherence in 2009 (Odds ratio [OR]=0.59; 95% CI: 0.40-0.85) and 2010 (OR=0.28, 0.13-0.59). EXEN adherence was associated with metformin use (OR=1.37; 1.27-1.48), endocrinologist care (OR=1.20; 1.14-1.26), or microvascular event (OR=1.20; 1.13-1.26) and hospitalization (OR=0.70; 0.64-0.76).

**Conclusion:** Adherence to EXEN or LIRA therapy is suboptimal and non-adherence to EXEN increased between 2005 and 2010. Adherence rate to

these GLP-1 therapies is lower in patients who do not achieve A1C goal than those who do. Medically treated nausea or vomiting and hospital admissions are contributing factors to non-adherence to EXEN therapy while endocrinologist care appears a mitigating factor. A new therapy with better tolerability may improve adherence hence patient outcomes.

Supported by: Sanofi

981

### Real-world characteristics of patients with type 2 diabetes on basal insulin initiating add-on GLP-1 analogue therapy

M.R. Dalal<sup>1</sup>, Z. Ling<sup>1</sup>, K.L. Davis<sup>2</sup>, J.L. Meyers<sup>2</sup>, A. DiGenio<sup>1</sup>;

<sup>1</sup>sanofi-aventis U.S., Bridgewater, <sup>2</sup>RTI Health Solutions, Research Triangle Park, USA.

**Background and aims:** There is limited real-world data on patients treated with basal insulins plus glucagon-like peptide-1 (GLP-1) analogs. This study aimed to document the real-world patient characteristics and outcomes of type 2 diabetes mellitus (T2DM) patients treated with basal insulin with and without add-on GLP-1 analog therapy.

**Materials and methods:** Using the General Electric Centricity electronic medical records (EMR) database, a retrospective analysis was conducted in T2DM patients aged ≥ 18 years who initiated a basal insulin between January 2005 and January 2012. Patients had ≥ 6 months EMR activity prior to their index date and were stratified based on whether or not they initiated a GLP-1 analog as add-on therapy within the available follow-up period. Study index date was the first insulin prescription date for patients without subsequent add-on therapy (INS-only) and the first date of combined prescription of both drugs for patients with an add-on therapy (INS + GLP). INS-only patients were used as a reference group. Primary study endpoints included HbA<sub>1c</sub> levels and body weight and were analyzed descriptively during a maximum 12-month follow-up period. Hypoglycemia rates and gastrointestinal related events will be evaluated later.

**Results:** 29,684 patients were eligible (INS-only: N = 28743, INS + GLP: N = 939). Baseline demographic and clinical characteristics are shown (Table). At time of insulin initiation, HbA<sub>1c</sub> levels were similar for both groups. Mean HbA<sub>1c</sub> levels were 8.89% for INS-only patients at insulin initiation and 8.45% for INS + GLP patients at GLP add-on index date. Mean baseline body weight was 97.22 and 109.06 kg for INS-only and INS + GLP patients, respectively (Table). Median time between first insulin initiation date and GLP add-on date for INS + GLP patients was 468 days. At 6 months and 1 year post index date, INS-only patients showed a significant reduction in HbA<sub>1c</sub> levels from baseline of -0.77% (P < 0.0001) and -0.67% (P < 0.0001), respectively. Body weight in the INS-only group was significantly lower from baseline at 6 months (-0.24 kg, P < 0.0001), but not at 1 year after index date (+ 0.14 kg, P = 0.018, Table). INS + GLP patients also showed a significant reduction in HbA<sub>1c</sub> levels from baseline (6 months: -0.37%, P < 0.0001; 1 year: -0.35%, P < 0.0001). Body weight in the INS + GLP group was significantly lower from baseline at both time points (6 months: -1.90 kg, P < 0.0001; 1 year: -1.97 kg, P < 0.0001, Table).

**Conclusion:** This study demonstrates that in a real-world setting, the addition of GLP-1 analog therapy to basal insulin in T2DM patients can be associated with improved glycemic control and reduction in body weight. Further analyses are needed to investigate the impact of possible confounders on outcomes.

Table: Demographic and clinical characteristics of INS-only and INS + GLP patients

	INS-only (n = 28,743)	INS + GLP (n = 939)
Mean (sd) age, years	60.1 (12.8)	55.0 (11.2)
≥ 65 years, n (%)	11,171 (38.9)	195 (20.7)
Male, n (%)	14,215 (49.5)	430 (45.8)
Mean (sd) CCI score	1.1 (1.6)	0.8 (1.3)
Mean (sd) baseline HbA <sub>1c</sub> , %	9.04 (2.02)	8.93 (1.80)
Mean (sd) baseline weight, kg	97.22 (24.92)	109.06 (24.62)
Mean (sd) change in HbA <sub>1c</sub> , %		
6 months	−0.77 (2.00)*	−0.37 (1.39)*
1 year	−0.67 (2.06)*	−0.35 (1.58)*
Mean (sd) change in body weight, kg		
6 months	−0.24 (7.17)*	−1.90 (7.41)*
1 year	+0.14 (7.86)†	−1.97 (7.11)*

CCI, Charlson Comorbidity Index. \* Within-group  $P < 0.0001$ , † within-group  $P = 0.018$

Supported by: Study funding and editorial support provided by sanofi-aventis U.S.

## 982

### Is HbA<sub>1c</sub> a reliable measure for assessing glycaemic control?

M. Schweitzer<sup>1</sup>, D.A. Cavan<sup>2</sup>, R. Ziegler<sup>3</sup>, I. Cranston<sup>4</sup>, C. Parkin<sup>5</sup>, R.S. Wagner<sup>6</sup>;

<sup>1</sup>Roche Diagnostics GmbH, Mannheim, Germany, <sup>2</sup>Royal Bournemouth Hospital, Bournemouth, UK, <sup>3</sup>Diabetes Clinic for Children and Adolescents, Muenster, Germany, <sup>4</sup>Queen Alexandra Hospital, Portsmouth, UK, <sup>5</sup>CGParkin Communications, Inc., Boulder City, USA, <sup>6</sup>Roche Diagnostics, Inc., Indianapolis, USA.

**Background and aims:** HbA<sub>1c</sub> is generally considered the “gold standard” for assessing glycaemic control in people with diabetes. However, discrepancies between HbA<sub>1c</sub> values obtained from different methodologies have been reported. We assessed the correlation of results between immunoassay (IM) methodologies and high-performance liquid chromatography (HPLC).

**Materials and methods:** We compared paired baseline HbA<sub>1c</sub> values from two large clinical trials involving poorly controlled type 1 (T1DM) and type 2 (T2DM) diabetes. In our first comparison, blood samples for paired HbA<sub>1c</sub> values were obtained from 453 poorly controlled T2DM subjects, drawn from a large, cluster-randomized, multicenter study. HbA<sub>1c</sub> values were obtained from point of care (POC) tests, using IM methodology (A1C NOW+, Bayer Diagnostics, Tarrytown, New York, USA) and HPLC methodology (Variant II and Variant II Turbo haemoglobin testing systems, Bio-Rad Laboratories, Hercules, California, USA), performed at a central laboratory. Average HbA<sub>1c</sub> values were  $8.4 \pm 1.4\%$  and  $8.6 \pm 1.2\%$  for IM and HPLC, respectively. Our second comparison looked at paired baseline HbA<sub>1c</sub> values from 193 poorly controlled T1DM and T2DM subjects enrolled in the Automated Bolus Advisor Control and Usability Study (ABACUS), a large, prospective, randomized, multi-national study of poorly controlled ( $\text{HbA}_{1c} \geq 7.5\%$  /  $58 \text{ mmol/l}$ ) T1DM and T2DM, MDI-treated subjects. We compared baseline results obtained at local laboratories (using several methodologies) and central laboratory values (VII Turbo haemoglobin testing system, Bio-Rad Laboratories, Hercules, California, USA). Mean HbA<sub>1c</sub> values were  $9.04 \pm 1.17$  (local laboratory) and  $8.85 \pm 1.15\%$  (central laboratory).

**Results:** Analysis of the first data set revealed that IM results were lower than HPLC values. Although mean bias between methods was found to be acceptable at  $-0.18\%$ , standard deviation was  $0.96\%$ , and there was a significant difference ( $p < 0.001$ ) between method results as indicated by a 95% limit of agreement of  $-0.27\%$  to  $-0.09\%$  HbA<sub>1c</sub> (95% confidence interval [95% CI]),  $r = 0.74$ . IM values differed from HPLC laboratory values by  $>0.5\%$  HbA<sub>1c</sub> in  $>59\%$  of cases and by  $>1.0\%$  HbA<sub>1c</sub> in  $>27\%$  of the cases. In the second analysis, we saw a significant difference between the methodologies ( $p < 0.0001$ ) with a positive bias of  $0.15$  in IM methodology, and limits of agreement from  $0.09$  to  $0.21$  (95% CI),  $r = 0.92$ .

**Conclusion:** Because many clinicians still rely primarily on HbA<sub>1c</sub> test results when making treatment decisions, it is important that HbA<sub>1c</sub> values taken are accurate in order to avoid under-treating or over-treating patients. Given the discrepancies found in our analysis of two different data sets, cli-

nicians may consider use of self-monitoring of blood glucose (SMBG) in a structured manner, utilizing 7-point glucose profiles obtained over 3 consecutive days, to confirm actual levels of glycaemic control. These 7-point glucose profiles have been shown to highly correlate with HbA<sub>1c</sub> levels obtained from HPLC methodologies. Moreover, use of structured SMBG can provide important and unique clinical information about patterns of intra-day glycaemia variability that cannot be obtained from HbA<sub>1c</sub> testing, alone.

Clinical Trial Registration Number: NCT01460446

Supported by: Roche Diagnostics, Inc.

## 983

### Relative and absolute contributions of postprandial and fasting plasma glucose over a 24hour period

R. Peter<sup>1</sup>, G. Dunseath<sup>2</sup>, S. Luzio<sup>2</sup>, D. Owens<sup>2</sup>;

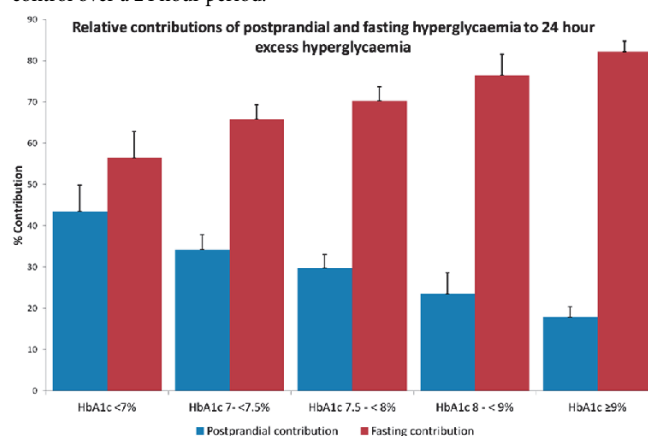
<sup>1</sup>Neath Port Talbot Hospital, UK, <sup>2</sup>Diabetes Research Group, Swansea University, UK.

**Background and aims:** To determine the relative and absolute contributions of postprandial glucose (PPG) and fasting/pre-prandial plasma glucose (FPG) over a 24 hour period and HbA<sub>1c</sub> respectively in persons with type 2 diabetes.

**Materials and methods:** Subjects ( $n = 52$ , 37 men) had 12 hour plasma glucose (PG) profiles determined in response to 3 serial, identical test meals, given at 0-240, 240-480 and 480-720, comprising the daytime period. The nighttime period was determined by extending the profile from the 720 minute value to the pre-breakfast value, corresponding to the 24 hour glucose level. The PPG exposure was calculated for each 4 hour meal period. Excess hyperglycaemia was calculated as the area above a PG concentration of  $5.5 \text{ mmol/L}$  for the whole 24 hour period. Fasting hyperglycaemia was the difference between the excess hyperglycaemia and the PPG exposure. Subjects were divided into 5 groups according to HbA<sub>1c</sub> (Gp1:  $<7\%$ , Gp2:  $7-7.5\%$ , Gp3:  $7.5-8\%$ , Gp4:  $8-9\%$ , Gp5:  $\geq 9\%$ ). The absolute contribution of PPG and fasting hyperglycaemia to excess HbA<sub>1c</sub> was also calculated.

**Results:** The relative contributions of PPG glucose exposure to 24hour excess hyperglycaemia for the 5 groups were 43.5, 34.2, 29.7, 23.5 and 17.8%, Gp 1-5 respectively ( $p = 0.004$ ) while the relative contributions of fasting hyperglycaemia increased from 56.5, 65.8, 70.3, 76.5 and 82.2%, from Gp1 to Gp5 respectively. Absolute contributions of PPG to excess HbA<sub>1c</sub> were not significantly different in the 5 groups, whilst the absolute contributions of fasting hyperglycaemia to excess HbA<sub>1c</sub> increased significantly as HbA<sub>1c</sub> increased.

**Conclusion:** The relative contributions of postprandial glucose to 24 hour excess hyperglycaemia still decreases as glycaemic control worsens, although fasting hyperglycaemia contributes substantially in all groups and increases as HbA<sub>1c</sub> deteriorates. However the absolute contribution of postprandial glucose to excess HbA<sub>1c</sub> does not differ significantly with varying glycaemic control over a 24 hour period.



## 984

**Rare, 'fast' haemoglobin variants may interfere with HbA<sub>1c</sub> measurement using ion exchange high performance liquid chromatography: implications for diagnosis of diabetes**S. Misra<sup>1</sup>, M.R. Hancock<sup>2</sup>, D. Roper<sup>2</sup>, B. Green<sup>3</sup>, R.A. Round<sup>4</sup>, R. Cramb<sup>4</sup>, S.E. Manley<sup>4</sup><sup>1</sup>Metabolic Medicine, Imperial Healthcare NHS Trust, London, <sup>2</sup>Clinical Biochemistry, Imperial NHS Trust, London, <sup>3</sup>Waters Corporation, Cheshire, <sup>4</sup>Clinical Biochemistry, University Hospital Birmingham Foundation NHS Trust, UK.

**Introduction:** Haemoglobin (Hb) variants are common and many are clinically silent. Ion exchange high performance liquid chromatography (IE HPLC) performed for HbA<sub>1c</sub> measurement often identifies variants, which, depending on their elution time can lead to over- or under-estimation of HbA<sub>1c</sub>. Most IE HPLC methods have been evaluated in the presence of common variants (e.g. Hb S & C). While most of the ~1500 variants detailed in the online Hb database are individually rare, they are cumulatively significant and a particular problem occurs if the variant or its glycated fraction co-elutes with HbA<sub>1c</sub>. Interference from undiagnosed Hb variants has a worrying impact when an absolute value of HbA<sub>1c</sub> is used to diagnose diabetes. We report 3 types of interference from Hb variants co-eluting with HbA<sub>1c</sub> on analyses performed using IE HPLC.

**Methods:** Cases presented to Clinical Biochemistry departments at our two hospitals. HbA<sub>1c</sub> was determined using Menarini-HA8160 or Tosoh G8 IE HPLC. Further analysis was performed using electrospray ionisation mass spectrophotometry (ESI-MS).

**Results:** Class 1: Distinct variant Hb's co-eluting with HbA<sub>1c</sub>. Variant Hb's were identified due to obviously abnormal chromatograms with 'error codes' generated by the analyser (figure 1). In each case the variant elutes with HbA<sub>1c</sub>. Hb Camperdown and K Woolwich have previously been shown to affect HbA<sub>1c</sub> analysis. However this is the first report of Hb J Iran interfering. These disproportionately large peaks can easily be identified. Class 2: Variants identified through asymmetry of the HbA<sub>1c</sub> peak. Asymmetry of HbA<sub>1c</sub> peaks was detected during analysis (figure 2). ESI-MS confirmed the presence of variants. Cases depict either a small peak eluting closely to HbA<sub>1c</sub> (Hb Belleville, Pyrgos and South Florida), or, a subtle shoulder to the normal HbA<sub>1c</sub> peak (Hb J-Bangkok, La Desirade and Riccarton). Analytical systems may not identify these anomalies, which if incorporated into the peak, can falsely increase HbA<sub>1c</sub>. Class 3: Undetectable variants, found by a discordant clinical picture. Figure 3 shows the chromatogram obtained from the blood of a 25-year-old lady exhibiting symptoms of tiredness. HbA<sub>1c</sub> was elevated at 152 mmol/mol (16.1%) (Menarini HA-8140), with a normal shaped peak. Fasting glucose performed was normal. ESI-MS revealed the patient was heterozygous for Hb Wayne, an alpha chain mutation. The true HbA<sub>1c</sub> was found to be 23mmol/mol (4.3%).

**Conclusion:** This case series highlights the heterogeneity with which fast Hb variants can interfere with HbA<sub>1c</sub> analysis. The classification system as outlined may help keep laboratory staff alert to potential interferences, which become critically important when using HbA<sub>1c</sub> for the diagnosis of diabetes. Though IE-HPLC is susceptible to interference, alternative methods (borate affinity chromatography / immunoassay) also have their limitations, which may not be easily recognisable. Requesting clinicians should be aware of the limitations of HbA<sub>1c</sub> analysis and query results discordant with the clinical picture. This is of even more importance when considering using HbA<sub>1c</sub> for the diagnosis of diabetes.

## 985

**Underutilisation of antihyperglycaemic combination therapy in eligible, treatment-naïve patients with type 2 diabetes mellitus**Y. Qiu<sup>1</sup>, A.Z. Fu<sup>2</sup>, M.J. Davies<sup>1</sup>, S.S. Engel<sup>1</sup><sup>1</sup>Merck Sharp & Dohme, Whitehouse Station, <sup>2</sup>Georgetown University Medical Center, Washington, USA.

**Background and aims:** Patients with type 2 diabetes mellitus (T2DM) are at increased risk for vascular morbidity and mortality. Glucose control with antihyperglycaemic agents has been shown to reduce the risk of microvascular and diabetes-related complications. The likelihood of reaching HbA<sub>1c</sub> treatment targets using monotherapy with antihyperglycaemic agents is low in T2DM patients with elevated HbA<sub>1c</sub> levels. Therefore, AACE/ACE guidelines recommend initiating dual oral combination therapy which includes metformin in untreated patients with an HbA<sub>1c</sub> of 7.6 to 9.0%. The purpose of

this analysis was to estimate the proportion of treatment-naïve patients with T2DM and an HbA<sub>1c</sub> of 7.6 to 9% who initiate antihyperglycaemic treatment with combination therapy and to determine patient-related factors associated with initiating combination therapy.

**Methods:** Using the US GE electronic medical records database, patients included in this analysis were those with a T2DM diagnosis from 2003 to 2010, at least one HbA<sub>1c</sub> measurement of 7.6 to 9% (first instance = index date), continuous enrollment in database for ≥12 months prior to and ≥6 months after index date, and no prescriptions for antihyperglycaemic agents in the 12 months prior to index date. Patients receiving prescriptions for any oral and/or injectable antihyperglycaemic agents as combination therapy (freely co-administered or fixed-dose included) or monotherapy within 30 days of index date were considered to have initiated therapy at index date. Logistic regression was performed to identify factors associated with initiating combination therapy.

**Results:** Of the 30,501 selected patients, 8% initiated antihyperglycaemic treatment with combination therapy and 36% initiated with monotherapy within 30 days of index date. Over the 2003 to 2010 time frame, the proportion of patients initiating any antihyperglycaemic monotherapy increased over time, but the proportion who initiated with combination therapy was relatively flat during this time period. After adjusting for baseline characteristics, patients with T2DM and an HbA<sub>1c</sub> of 7.6 to 9.0% were more likely to be prescribed combination antihyperglycaemic therapy if they had higher HbA<sub>1c</sub> (adjusted odds ratio [OR] = 1.78 [95% CI: 1.61, 1.97]). Patients were less likely to be prescribed combination antihyperglycaemic therapy if they were older (per 5-year OR = 0.95 [0.92, 0.97]), lived in regions outside of the Southern US (ORs ranged from 0.44 - 0.68 for different regions compared to South), or had chronic renal disease/renal failure (OR [95% CI] = 0.46 [0.28, 0.76]).

**Conclusion:** In a cohort of untreated patients meeting guideline recommendations for initiating combination antihyperglycaemic therapy, a low proportion of patients initiated with a broad range of combination therapies (8%) or any antihyperglycaemic regimen (44%). Greater efforts are needed to address underutilisation of antihyperglycaemic agents and the appropriate use of combination antihyperglycaemic therapy.

Supported by: Merck Sharp &amp; Dohme Corp.

## 986

**Adding a 3rd agent to 2 oral antidiabetic drugs (OADs) - real-world economic impact and effect of treatment persistence on clinical outcomes**O. Baser<sup>1</sup>, S. Zhou<sup>2</sup>, W. Wei<sup>2</sup>, Z. Ling<sup>2</sup>, L. Xie<sup>1</sup>, P. Levin<sup>3</sup><sup>1</sup>STATinMED Research, Ann Arbor, <sup>2</sup>sanoofi-aventis U.S., Bridgewater,<sup>3</sup>MODEL Clinical Research, Baltimore, USA.

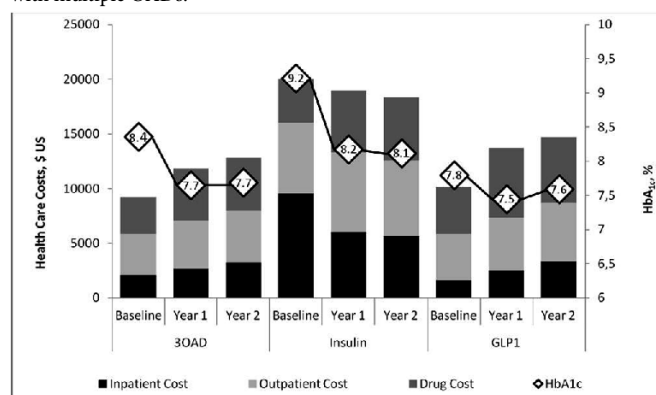
**Background and aims:** Patients with type 2 diabetes mellitus (T2DM) may need to intensify treatment when failing to maintain glycemic control with OADs only. This study assessed real-world clinical and economic outcomes, and the impact of treatment persistence, of adding a 3rd OAD (3OAD), a glucagon-like peptide 1 (GLP1) agonist (+GLP1), or insulin (+Insulin) to 2 OAD regimens.

**Materials and methods:** This retrospective cohort study used IMPACT<sup>®</sup>, a national managed care claim database, from 2000-2011. Included were adult T2DM patients previously on 2 OADs and initiating a 3rd OAD, GLP1, or insulin, with continuous health care coverage 6 months before (baseline) and 2 years after (follow-up) initiation. Within each cohort (3OAD, +GLP1, or +Insulin), we examined HbA<sub>1c</sub> and annual health care costs during baseline and the 1st and 2nd year of follow-up. We also assessed the association between HbA<sub>1c</sub> reduction from baseline and treatment persistence (ie, remaining on the initiated 3rd agent treatment class [OAD only, insulin, or GLP1] at the end of 1- or 2-year follow-up).

**Results:** A total of 51,771 patients (mean age 55.6 years, male 60.0%) were included (3OAD: 41052 [79.3%]; +Insulin: 6904 [13.3%]; +GLP1: 3815 [7.4%]). The 3 cohorts were significantly different at baseline, including HbA<sub>1c</sub> levels ( $P < 0.001$ ; Figure). During follow-up, annual drug costs increased in all 3 groups. However, +Insulin patients had decreased total costs (92% of baseline), mainly due to decreased inpatient costs (59% of baseline), while total and inpatient costs increased for 3OAD (139 and 155%) and +GLP1 (144 and 213%; Figure). At the end of 2-year follow-up, HbA<sub>1c</sub> reduction was -0.88% in +Insulin, -0.64% in 3OAD, -0.33% in +GLP1, among those with HbA<sub>1c</sub> data available (Figure). During follow-up, 2-year persistence rate was 72% in 3OAD, 57.1% in +Insulin, and 35.6% in +GLP1. In those with HbA<sub>1c</sub> data available, for +Insulin patients, 2-year treatment persistence was associated with greater HbA<sub>1c</sub> reduction, compared with switching during the 1st year (-0.99 vs



–0.59%,  $P = 0.032$ ); the opposite was observed for 3OAD (–0.65 vs –0.92%,  $P = 0.009$ ). No difference was observed in +GLP1 (–0.48 vs –0.23%,  $P = 0.128$ ). **Conclusion:** This real-world study showed that most T2DM patients added a 3rd OAD, instead of insulin or GLP1 after failing 2 OADs. All patients had HbA<sub>1c</sub> reduction during 2-year follow-up, but only the +Insulin group had decreased HbA<sub>1c</sub> and decreased total health care costs. Both 3OAD and +GLP1 groups had significant increase in health care costs, due to increased inpatient and drug costs. Treatment persistence was associated with better HbA<sub>1c</sub> reduction in the +Insulin group; the opposite was observed in the 3OAD group. This data supports the call for timely intensification and persistent use of insulin among T2DM patients not maintaining glycemic control with multiple OADs.



Graph: Health care costs (left Y-axis, bars) and HbA<sub>1c</sub> levels (right Y-axis, diamonds) at baseline, 1-year follow-up, and 2-year follow up, for patients from the total cohort adding a 3rd oral, insulin, or GLP1 to 2 OADs.

Supported by: Study funding and editorial support provided by sanofi-aventis U.S.

## 987

### The role of work related factors to glycaemic control in employees with diabetes

A.M. Mansinho<sup>1</sup>, C.L. Oliveira<sup>1</sup>, C.P. Paulo<sup>1</sup>, G.D. Alexandre<sup>1</sup>, I.M. Pereira<sup>1</sup>, J.F. Azevedo<sup>1</sup>, M. Almeida<sup>1</sup>, J.F. Raposo<sup>1,2</sup>

<sup>1</sup>Public Health Department, New University of Lisbon, <sup>2</sup>Diabetes Portugal, Lisbon, Portugal.

**Background and aims:** The work environment seems to influence the level of adaptation of employees with diabetes. Nevertheless little research exists. A bad disease adaptation can cause a greater absenteeism among people with diabetes. Our aim is to describe which work factors could influence the glycaemic control and evaluate the impact of Diabetes on the work ability.

**Materials and methods:** Descriptive cross-sectional and observational study. The clinical data was collected by a self-completed survey instrument. The work-related factors were collected using the Job Stress Scale (JSS) with 3 dimensions (demand, control and social support). Participants and settings: People with type 1 or type 2 diabetes employees (n=101) aged 18 to 67 years attending a portuguese diabetes center during 2 weeks of March.

**Results:** The mean age was 45.8 (±12.5) years (DM1 39.6; DM2 52.4), 70.3% were male; 67.3% had less than 12 years of education, the average duration of diabetes was 13.3 (±8.4) years. Fifty-two (51.5%) had DM1 and 49 (48.5%) DM2. The duration of diabetes was longer in DM1 (16.7±12.5y) than in DM2 (9.7±5.1y) ( $p=0.00$ ). Concerning JSS, higher level of demand score (14.8) control (18.7) and social support (20.6) suggested better metabolic control HbA<sub>1c</sub> (8.0%). Sixteen percent (n=16) indicated professional dissatisfaction of which 87.5% had a worse metabolic control. To note that 16% of diabetics did not inform their supervisors that they had diabetes. Thirty patients (29.7%) modified some of their work tasks and 26 (25.7%) interrupted some of them. People with type 1 diabetes evidenced an average absenteeism of 5.3 days, in the last 3 months, versus a 1.9 average for type 2 diabetes. Patients with complications associated to the disease missed their work, an average of 7.2 days against 1.3 of the ones with no complications.

**Conclusion:** The results suggest that people with diabetes, higher education, increase demand, control and social support at work could have better metabolic control. Some work factors such as satisfaction seem to interfere with glycaemic control. Diabetes is a chronic disease whose physical and psychological adaptation to the work environment is a key factor to retrieve them

to the labour market. Future work should corroborate some of these findings. On the other hand it will be important to develop worksite interventions to facilitate the proper and adequate integration of people with diabetes in their workplace to reduce absenteeism.

## 988

### The association between income and long term glycaemic control

S.D.M. Bot<sup>1</sup>, L. Kingo<sup>2</sup>, E. van 't Riet<sup>2</sup>, J.M. Dekker<sup>2</sup>, G. Nijpels<sup>1</sup>

<sup>1</sup>Department of General Practice, <sup>2</sup>Department of Epidemiology and Biostatistics, EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands.

**Background and aims:** It is well known that low socio economic status (SES) is associated with adverse diabetes outcomes. Most studies examining the association between SES and diabetes outcomes have used a cross-sectional design. In this study we present longitudinal data on the association between income, as indicator of SES, and long term changes in HbA<sub>1c</sub> using patient data of a structured diabetes care system in the Netherlands.

**Materials and methods:** Patients with type 2 diabetes who entered a diabetes care system in the Netherlands between 1998 and 2010 were followed annually for up to 12 years. SES was based on census data on average household income using patient postal code data, and median income tertiles were derived. Baseline differences across the three income groups were compared using  $\chi^2$  analysis for dichotomous variables and linear regression for continuous variables. Linear mixed model analyses were performed to compare differences in mean HbA<sub>1c</sub> between income groups during follow-up using SPSS 17.

**Results:** Income data were available of 7571 patients of a total of 8245 patients who entered the diabetes care system during the study period. At entry, demographic and clinical characteristics differed significantly across income groups with higher age (mean 66 years ± 12.6 vs mean 60 years ± 11.3;  $p<0.001$ ), more women (59% vs 42%;  $p<0.001$ ), and longer duration of diabetes (mean 4.0 ± 6.6 vs 2.8 ± 5.2;  $p<0.001$ ) in the lowest income group compared to the highest income group. In Figure 1, the course of HbA<sub>1c</sub> for the three income groups is shown. Mean difference in HbA<sub>1c</sub> over 12 years, adjusted for age and sex, was 0.20 (95% CI, 0.14 to 0.26) between the highest and lowest income group. This difference attenuated to 0.14 (95% CI, 0.08 to 0.20) when additionally adjusted for diabetes duration and year of entry.

**Conclusion:** Diabetes patients with a lower income are diagnosed at a higher age. Despite equal access to care for all patients, income had a significant impact on long term glycaemic control in this large-scale population of type 2 diabetes patients.

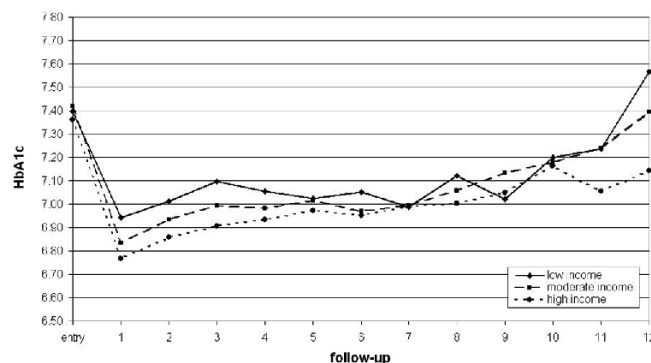


Figure 1. Mean HbA<sub>1c</sub> per income group during up to 12 years follow-up (low: median monthly income of 1300 euro; moderate: 1700 euro; high: 2200 euro)

## PS 082 Diabetes education

989

### A new web site hosting serious games to promote education for flexible insulin therapy, insulin pump and continuous glucose monitoring

M. Joubert, A. Guillaume, Y. Reznik;

Endocrinology Unit, University Hospital of Caen, France.

**Background and aims:** The intensified insulin therapy with multiple daily injections (MDI) or continuous subcutaneous insulin injection (CSII) is the gold standard for treatment of type 1 diabetes. In addition, education to flexible insulin therapy (FIT) can improve dietary freedom and quality of life while maintaining good glycemic control. In recent years, personal continuous glucose monitoring (CGM) has emerged as an additional technology to improve type 1 diabetes control. These technological devices (CSII and CGM) and the FIT method need to be implemented with patients as part of therapeutic education programs that are time consuming for caregivers. This last point represents a limitation for the spreading of these therapeutic modalities. To address this problem, we developed serious games to be used as educational tools for CSII, CGM and FIT.

**Materials and methods:** These serious games have been developed by a multi professional team including diabetes physicians, therapeutic education physicians, nutritionists, diabetes nurses, game designers and computer scientists. These interactive media are hosted on a website in French and English language in free access. There are 3 games dedicated to children, adolescents and young adults.

**Results:** The two major serious games are “Birman Case” and “Time-Out”. These are adventure games starring a hero with type 1 diabetes using the FIT method in MDI or CSII (with or without CGM option). The player have to help the main character to manage his diabetes during the game. These games are enhanced with a glycemic variation simulator that makes them realistic and truly interactive. The simulator of “Time-Out” is based on a validated metabolic model and operates in accelerated real-time. Several situations that can induce hypo- or hyperglycemic state are imposed to the player during the game: meals containing various amount of carbohydrates, unplanned physical activity, alcohol absorption, pump failures... The player will have to manage these situations using insulin boluses, basal rate adaptation, carbohydrate intake... A summary of the therapeutic behavior of the player appears at the end of each game and can be exported to the physician. This report might be used as an evaluation and educational support. A multicentric french clinical study has begun to evaluate the impact of these games on type 1 diabetes patients knowledge and behaviour.

**Conclusion:** These educational tools are not a substitute for an education program offered by a team of health professionals but they can be integrated in such programs for the initiation, initial education, strengthening or evaluation.

990

### My diabetes my way: empowering people with diabetes through electronic record access

S.G. Cunningham<sup>1</sup>, R.R. McAlpine<sup>1</sup>, M. Brillante<sup>1</sup>, L. Wilson<sup>1</sup>, A. Waller<sup>2</sup>,

A. Emslie-Smith<sup>3</sup>, J. Walker<sup>4</sup>, A. Morris<sup>5</sup>, D. Wake<sup>5</sup>;

<sup>1</sup>Clinical Technology Centre, University of Dundee, <sup>2</sup>School of Computing, University of Dundee, <sup>3</sup>Diabetes Support Centre, Ninewells Hospital, Tayside Diabetes Managed Clinical Network, Dundee, <sup>4</sup>St John's Hospital, NHS Lothian, Livingston, <sup>5</sup>Clinical Research Centre, University of Dundee, UK.

**Background and aims:** My Diabetes My Way (MDMW - [www.mydiabetes-myway.scot.nhs.uk](http://www.mydiabetes-myway.scot.nhs.uk)) is the official NHS Scotland information portal, containing validated educational materials for people with diabetes and their carers. In December 2010 a new service was launched, allowing patients from across the whole of Scotland access to their shared electronic medical record. The source data is collected by the national diabetes system, Scottish Care Information - Diabetes Collaboration (SCI-DC). We aimed to analyse the first year of usage and uptake.

**Materials and methods:** A system was developed, containing information collected from primary care, secondary care, specialist screening services and laboratory systems across Scotland. It includes key diagnostic information; demographics; laboratory, lifestyle, foot and eye screening results; prescribed medication and clinical correspondence. This information was made available from the NHS Scotland 'shared diabetes record'. Changes can be tracked

over time using 'history' graphs and tables. Data items link to detailed descriptions, explaining why they are collected, what they are used for and what 'normal' values are. Tailored information links refer individuals to further facts related to their condition.

**Results:** 224 people with diabetes across all Scotland's 14 health boards have now accessed their own information. During the first year, 160 users accessed the system (most logins=164), with 1425 logins in total (average=8.9/patient; median=4). Audit trails show 19497 page views (122/patient), with laboratory 'test results' proving the most popular (3219 accesses;20/patient). The most utilised history graph was, unsurprisingly, HbA1c (793 accesses;5/patient). Feedback: “The knowledge provided helps me understand the normal parameters and where I stand/can improve”.

**Conclusion:** Although there are other patient access systems available worldwide, the MDMW system is unique as it provides access to information collected from all diabetes-related data sources. The system supports the self-management agenda of the Scottish Government and the objectives of the NHS Scotland Healthcare Quality Strategy. It is person-centred as it involves the individual in their care by allowing them to become partners in their clinical data monitoring. It is “timely” by providing rapid access to contemporary clinical results and is “equitable” by being available to all.

*Supported by: Scottish Diabetes Group*

991

### Who is using Twitter to communicate about diabetes and what for?

G. Gimenez-Perez<sup>1</sup>, M. Robert-Vila<sup>2</sup>, A. Recasens<sup>1</sup>, O. Simó<sup>1</sup>, I. Castells<sup>1</sup>;

<sup>1</sup>Diabetes, Endocrinology and Nutrition Unit, Hospital General de Granollers, Barcelona, <sup>2</sup>Institut Catala de la Salut; ABS Moli Nou, Sant Boi de Llobregat, Spain.

**Background and aims:** Web 2.0 tools have rapidly changed the way people communicate, and can potentially change health provider-patient interactions. Twitter is a widely used web 2.0 microblogging system using messages of less than 140 characters, which can be read by all twitter users. Positioning a hashtag (#) before the relevant words is used by tweet publishers to highlight tweet subject and facilitate its finding. The aim of this study was to analyze tweets and Twitter publishers related to diabetes in order to explore its potential as a communication tool between diabetes health providers and patients.

**Materials and methods:** All tweets under #diabetes published during periods of 1 hour (coordinated universal time (UTC) 1, 6, 11, 16 and 21 hours) of two working days were selected and stored under the favorites section of an on purpose Twitter address (@web20diabstudy) until further analysis. Tweet's origin, content and links were assessed by 2 investigators. Differences were solved by agreement. We also selected, using a list of random numbers, 100 out of the first 500 twitter addresses which displayed the word “diabetes” or “diabetic” in their description and evaluated the type of publisher behind the address.

**Results:** A total of 446 tweets were retrieved. 342 (77%) were English written and were further analyzed. 145 (42%) came from personal addresses (44% patients or relatives; 16% professionals; 40% others). 138 (40%) were from corporate addresses (64% private companies; 23% non-profit organizations; 13% healthcare organizations). The remaining (59; 18%) were from news and medical websites or others. On a whole 42 (12%) came from a health care provider (organization or professional). 260 (76%) of tweets offered a link to an additional source, mainly webpages or blogs (57%). Tweet's subjects were quite uniformly distributed with 73 (12%) tweets about treatment, 57 (16%) about personal experiences and 51 (15%) about diet. 53% of tweets published by patients or relatives related to personal experiences. In 167 (49%) tweets an intention of branding or selling was evident either in the tweet itself or in the publisher's address. Non-profit organizations and patients accounted for 56% (28% each) of the Twitter addresses evaluated, with only 4% belonging to healthcare professionals or organizations.

**Conclusion:** Twitter is a useful tool to identify patients with diabetes and therefore to explore their personal experiences and thoughts. Although Twitter might allow the communication between diabetes healthcare providers and patients with diabetes, at present healthcare providers are underrepresented in the Twitter world. Branding yourself or indirectly selling products is one of the main activities seen under #diabetes.

## 992

**The effect of a self-management oriented education and treatment programme (PRIMAS) for type 1 diabetic patients**

**B. Kulzer**, N. Hermanns, D. Ehrmann, N. Bergis, T. Haak;  
Research Institute of Diabetes Academy Mergentheim (FIDAM), Bad  
Mergentheim, Germany.

**Background and aims:** In a randomised, multi-centre trial, the effect of a structured education and treatment programme to promote self-management and empowerment for type 1 diabetic patients (PRIMAS) was compared to an established education programme (Structured education and treatment programme for type 1 diabetic patients) as an active comparator condition (ACC). Besides optimizing insulin treatment by testing basal insulin doses and carbohydrate factors as well as avoiding acute and late complications the PRIMAS programme also aims at motivational factors and the daily routine of living with diabetes.

**Materials and methods:** A total of 160 patients with type 1 diabetes recruited in diabetologist practices (PRIMAS: age  $45.9 \pm 13.8$  yrs, diabetes duration  $19.6 \pm 12.8$  yrs., 38.3% female, HbA1c  $8.3 \pm 1.1\%$ , 23.5% with CSII vs. ACC: age  $45.2 \pm 13.4$  yrs, diabetes duration  $19.4 \pm 13.2$  yrs., 49.4% female, HbA1c  $8.1 \pm 0.9\%$ , 27.8% with CSII) were randomised to either PRIMAS or the ACC. Main outcome was the HbA1c 6 months after participation in the respective programme. Secondary outcomes were diabetes related distress (Diabetes Distress Scale 17 items), extent of self-perceived empowerment (Empowerment scale 11 items), self-efficacy for diabetes management (Self-efficacy scale 10 items), diabetes knowledge (Knowledge test 11 items), and self-care behaviour (Summary of Diabetes Self Care Activity Scale). Baseline adjusted results are reported as mean  $\pm$  SEM.

**Results:** Baseline adjusted HbA1c was significantly more improved in PRIMAS than in the ACC ( $-0.33 \pm 0.1\%$  vs.  $-0.03 \pm 0.1\%$ ,  $p=.028$ ) at 6 month follow-up. Also patients treated with PRIMAS reported lower baseline adjusted diabetes related distress levels (mean item score  $0.9 \pm 0.1$  vs.  $1.1 \pm 0.1$ ,  $p=.049$ ), higher empowerment ( $27.4 \pm 0.5$  vs.  $26.0 \pm 0.5$   $p=.027$ ) and self-efficacy levels (mean score:  $23.3 \pm 0.4$  vs.  $21.9 \pm 0.4$   $p=.044$ ) than patients in the ACC. Satisfaction with insulin treatment was also higher after 6 month in PRIMAS than in the ACC (mean score:  $27.1 \pm 0.6$  vs.  $25.3 \pm 0.6$ ;  $p=.028$ ). Effects on knowledge (mean score  $7.7 \pm 0.2$  vs.  $7.9 \pm 0.2$   $p=.555$ ) and self-care behaviours (mean score:  $3.7 \pm 0.1$  vs.  $3.8 \pm 0.1$ ;  $p=.846$ ) did not differ significantly between the education programmes nor did insulin dose ( $0.66 \pm 0.02$  vs.  $0.63 \pm 0.02$  IU/kg;  $p=.284$ ).

**Conclusion:** PRIMAS is more effective in lowering HbA1c than the established education programme in type 1 diabetic patients. In addition, PRIMAS has proven its efficacy regarding the improvement of empowerment, self-efficacy and satisfaction with insulin treatment as well as regarding the reduction of diabetes related distress. The effects on knowledge and self-care behaviour are similar to those of the established education programme. Thus, PRIMAS provides a good alternative for treatment and education of type 1 diabetic patients.

Clinical Trial Registration Number: NCT 01220557

Supported by: Berlin Chemie AG

## 993

**Impact on diabetes behaviours: knowing versus guessing blood glucose values**

**S.V. Edelman**<sup>1,2</sup>, J. Pettus<sup>1</sup>, J. Greer<sup>2</sup>, P. Stenger<sup>3</sup>, H.C. Schachner<sup>3</sup>, N. Dunne<sup>3</sup>, J.L. Parkes<sup>3</sup>, S. Pardo<sup>3</sup>;

<sup>1</sup>Division of Endocrinology, Diabetes and Metabolism, University of California at San Diego, <sup>2</sup>Taking Control Of Your Diabetes, Del Mar, <sup>3</sup>Bayer HealthCare LLC, Diabetes Care, Tarrytown, USA.

**Background and aims:** Research suggests that some people with diabetes use perceptions of their blood glucose (BG) levels rather than BG testing to make decisions about their diabetes management. This study was conducted to assess the difference between self-reported, estimated BG values and BG values as measured on a BG meter. Another objective of the study was to obtain information on the perceptions that people with diabetes have about BG testing and the impact of knowing their BG value on their diabetes management.

**Materials and methods:** Subjects aged  $\geq 18$  years with type 2 diabetes (N = 297) attending 1 of 2 Taking Control of Your Diabetes (TCOYD; 501c3) conferences were asked to take a pre-fingerstick questionnaire about their diabetes management. Subjects were then asked, "What do you think your blood sugar level is now?" Study staff then performed a fingerstick to measure

the subject's BG value on a BG meter. Subjects were advised of their BG value and were asked to respond to additional statements related to their BG testing practices on a post-fingerstick questionnaire.

**Results:** On the pre-fingerstick questionnaire, the majority of subjects either strongly agreed, agreed, or neither agreed/disagreed with the statement, "My body tells me without testing if my blood sugar is low or high" (77%) and made decisions about their diabetes, such as insulin dosing, without testing on a BG meter (71%; Table 1). However, nearly half (46%) of subjects estimated BG values that were outside current ISO accuracy guidelines (ie, more than  $\pm 15$  mg/dL or  $\pm 20\%$  of meter glucose values  $<75$  and  $\geq 75$  mg/dL, respectively); 58% estimated BG values that were outside proposed more stringent accuracy guidelines (ie, more than  $\pm 15$  mg/dL or  $\pm 15\%$  of meter glucose values  $<100$  and  $\geq 100$  mg/dL, respectively). On the post-fingerstick questionnaire, nearly all subjects reported that knowing their blood sugar level by checking could help them make different diabetes decisions (99%), give them more confidence in their ability to manage their diabetes (98%), help them prevent low BG (98%), help them recognize and treat low BG (98%), and give them a better understanding of how food affects their BG level (98%; Table 1). In addition, 99% of subjects responded that they would make different decisions about their meals/snacks if they knew their BG by checking on a BG meter. Among subjects taking insulin (n = 86), 98% felt that checking their BG on a meter could give them more confidence in adjusting their daily insulin dose.

**Conclusion:** These findings suggest that regular self-monitoring of BG versus guessing can contribute a significant impact on diabetes management behaviors in people with diabetes.

**Table 1. Subject Questionnaire Results**

Table 1. Subject Questionnaire Results							
Statement	Number of subject responses (N = 294)						N, A, or SA*
	SA	A	N	D	SD	N/A	
<b>Pre-fingerstick questionnaire</b>							
My body tells me without testing if my blood sugar is low or high	83	127	34	49	19	2	77%
I make decisions about my diabetes, such as my food intake or my insulin dose even when I do not test my blood sugar	43	154	11	58	25	3	71%
<b>Post-fingerstick questionnaire</b>							
<b>Knowing my blood sugar by checking</b>							
Could help me make different diabetes decisions	172	113	5	3	1	0	99%
Could give me more confidence in my ability to manage my diabetes	176	107	6	4	1	0	98%
Could help me prevent low blood sugar	162	112	18	2	3	7	98%
Could help me recognize and treat low blood sugar	176	107	6	4	1	0	98%
After I eat could give me a better understanding of how food affects my blood sugar levels	162	112	18	2	3	7	98%
Gives me more confidence in adjusting my daily insulin dose	48	29	7	1	1	205	98%
<b>I would make different decisions in my meals/snacks if I knew my blood sugar by checking</b>	164	115	6	2	1	6	99%

SA, strongly agree; A, agree; N, neutral; D, disagree; SD, strongly disagree; N/A, not applicable.

\*Responses of N/A were not included in the total responses for the purposes of the percentage calculation.

Clinical Trials Registration Number: NCT01453413

Supported by: Bayer HealthCare LLC, Diabetes Care

## 994

**A meta-analysis of pedometer-based care on physical activity and health outcomes in type 2 diabetes mellitus**

**S. Qiu**, X. Cai, B. Yang, Z. Sun, J. He;

Department of Endocrinology, Zhongda Hospital, Institute of Diabetes, Medical School, Southeast University, Nanjing, China.

**Background and aims:** Pedometers have recently been widely used as a tool for motivating and monitoring physical activity (PA) in type 2 diabetes (T2DM). However, the concrete effects of pedometer-based care on diabetes education and management are unknown. Therefore, we aimed to conduct a meta-analysis of randomised controlled trials (RCTs) in order to evaluate the effectiveness of pedometer-based care on PA and health outcomes in T2DM.

**Materials and methods:** Search strategy MEDLINE, EMBASE and Cochrane Library from 1994 to 29 February 2012 were searched. And the related references of all articles collected were checked to make sure that no relevant suitable studies were missed. Selection criteria All RCTs in English-language were included if they made evaluations of pedometer-based care intervention using pedometer as a motivational tool to increase PA in T2DM with a reported change in steps per day assessed by pedometers. Data collection and analysis Two investigators independently selected trials, assessed trial quality and extracted data. Continuous data were calculated as mean differences (MD). Random-effects model and fixed-effects model were used to perform meta-analysis for with and without heterogeneity respectively.



**Results:** Only six articles containing 10 RCTs were available for pooling involving 597 participants. Compared with the control group, the daily step count in the pedometer-based care intervention group was significantly increased by 1782 over baseline (95% confidence interval (CI) 643 to 2920;  $P<0.0001$ ), and the body mass index (BMI) was decreased by 2.46 (95% CI, -3.55 to -1.37;  $P<0.0001$ ). However, there were no statistically significant improvements in glycated haemoglobin (HbA<sub>1c</sub>) (-0.19%, 95% CI -0.41% to 0.03%;  $P=0.09$ ), total cholesterol (TC) (-0.17 mmol/L, 95% CI -0.34 mmol/L to 0.00 mmol/L;  $P=0.06$ ) or diastolic blood pressure (-1.21 mmHg, 95% CI -2.79 mmHg to 0.37 mmHg;  $P=0.13$ ).

**Conclusion:** Although our overall results suggest that pedometer-based care has an association with significant increases in PA and significant decreases in BMI, we find no statistically significant improvements in HbA<sub>1c</sub>, TC or diastolic blood pressure and additional RCTs are needed on these topics.

## 995

### The 'piecing together diabetes' education tool improves healthcare professional knowledge and inpatient diabetes care

R. Herring<sup>1</sup>, C. Pengilly<sup>1</sup>, H. Hopkins<sup>1</sup>, B. Tuthill<sup>1</sup>, N. Patel<sup>2</sup>, C. Nelson<sup>3</sup>, A. Currie<sup>4</sup>, D. Russell-Jones<sup>1</sup>

<sup>1</sup>Centre for Endocrinology, Diabetes and Research, Royal Surrey County Hospital, Guildford, <sup>2</sup>St Georges Hospital, London, <sup>3</sup>Northern General Hospital, Sheffield, <sup>4</sup>Hillingdon Hospital, Middlesex, UK.

**Background and aims:** Twenty percent of the United Kingdom's inpatient population have diabetes. In 2009, the National Diabetes Inpatient Audit identified that healthcare professionals often lack the confidence and knowledge to manage this patient group appropriately. We developed a novel inter-professional diabetes educational tool for use in the hospital environment. The jigsaw design incorporates all aspects of inpatient care from admission to discharge. Diabetes specialists facilitate learning through a combination of discussions, skills and activities.

**Materials and methods:** The education tool was piloted in four public hospitals in the United Kingdom. Thirty-one healthcare professionals were educated: 3 pharmacists, 17 nurses, 8 healthcare assistants and 3 junior doctors. The educational intervention was evaluated using Kirkpatrick's model, which assessed the learner's reaction, learning and behaviour and clinical outcomes related to key areas of inpatient diabetes care.

**Results:** Healthcare professional's confidence improved from 58% to 94% ( $p=0.002$ ) and knowledge improved from  $12.4\pm0.6$  to  $15.0\pm0.6$  (mean  $\pm$  SEM,  $p=0.005$ ). Appropriate blood glucose monitoring improved from 67% to 92% ( $p=0.026$ ). Management errors were reduced from 74% to 44% ( $p=0.045$ ) with appropriate hypoglycaemia management improving from 9% to 75% ( $p=0.003$ ). The number of patients with documented foot assessment improved from 15% to 33% ( $p=0.036$ ). Improvement in the number of appropriate diabetes referrals and reduction in prescribing errors did not reach statistical significance.

**Conclusion:** The well designed inpatient-teaching tool can be delivered effectively to healthcare professionals. The adaptability of the modules both in time and content made it user friendly. Widespread application of this tool could provide hospital staff with a robust, flexible and validated education tool, improve patient safety and potentially reduce inpatient care costs.

*Supported by: The educational toolkit has been produced in partnership with Lilly*

## 996

### Adherence to diabetes guidelines is a matter of behaviour of physicians rather than their knowledge levels

C. Yilmaz<sup>1</sup>, I. Satman<sup>2</sup>, S. Imamoglu<sup>3</sup>, on behalf of ADMIRE Study Group; <sup>1</sup>Internal Medicine, Endocrinology & Metabolism, Ege University Faculty of Medicine, Izmir, <sup>2</sup>Internal Medicine, Endocrinology & Metabolism, Istanbul University, Istanbul Faculty of Medicine, <sup>3</sup>Internal Medicine, Endocrinology & Metabolism, Uludag University Faculty of Medicine, Bursa, Turkey.

**Background and aims:** Clinical practice guidelines on diabetes mellitus (DM) have been revised in 2009 by The Society of Endocrinology & Metabolism, Turkey. The ADMIRE Project is designed to evaluate the effect of implementation activities to increase physicians' awareness on guidelines. The aim of this abstract is to determine relationship between the knowledge level about the recommendations in guidelines and the level of adherence of physicians to guidelines during follow-up of diabetes patients in Turkey.

**Materials and methods:** The study had two phases: "retrospective" and "prospective." Here we evaluated the prospective phase. In the initial period, medical records of 885 T2DM patients of 175 physicians were kept for 6 months, previous to awareness activities (PRE). Then, following a series of activities with the intention to increase the awareness of physicians on DM guidelines (POST), medical records of another group of 1,616 T2DM patients were kept for 6 months. In POST, physicians were asked to complete a 20 items questionnaire to measure their knowledge level (scored over 100). Adherence to guidelines was evaluated for two facets of patient management: (1) adherence to follow-up procedures (ADH\_FU), with three subdomains for medical history (HIST), physical examination (PE) and laboratory evaluation (LAB) (2) and adherence to treatment decisions with regards to management of patients with antidiabetic, antihypertensive and antilipid medications. The adherence scores between PRE and POST were compared, and the correlation coefficients between knowledge score and adherence scores were calculated.

**Results:** Physicians' average total score of knowledge on guidelines were 54.6. Their mean adherence scores, over 10, in PRE and POST were as follows: HIST 8.17 vs. 8.90 ( $p=0.022$ ), PE 4.79 vs. 7.12 ( $p<0.001$ ), LAB 6.97 vs. 6.07 ( $p<0.001$ ) and ADH\_FU 66.5 vs. 73.6 ( $p<0.001$ ), respectively. The percent of patients who were given antidiabetic treatment in agreement with the guidelines in PRE and POST were similar (50% vs 54%, respectively,  $p=0.22$ ). However, the percent of patients who were given antihypertensive treatment (79% vs. 87%,  $p=0.001$ ) and antilipid treatment (87% vs. 93%,  $p=0.005$ ) in agreement with the guidelines increased significantly in POST when compared to PRE. Correlation coefficients ( $r$ ) between the score of knowledge and ADH\_FU score and the percent of patients treated in agreement with the guidelines, were found to be between -0.093 and +0.152 (all  $p$  values  $>0.05$ ).

**Conclusion:** Adherence of physicians to DM guidelines has shown an increase in many aspects of patient management following the implementation of awareness activities. However, surprisingly, no correlation could be detected between the knowledge of physicians and adherence to guidelines. This finding led us to consider that, the level of adherence to guidelines is a matter of behavior rather than a matter of knowledge. Therefore, we suggest that, activities targeting to increase the adherence to guidelines should focus not just to increase the knowledge, but mainly to change the attitude of physicians.

## 997

### Evaluation of the effectiveness of a 10-day structured diabetes education program for geriatric nurses - a prospective controlled trial

A. Bahrman<sup>1</sup>, E. Hölscher<sup>2</sup>, K. Hodeck<sup>3</sup>, S. Weyerer<sup>4</sup>, P. Oster<sup>2</sup>, W.G. Daniel<sup>1</sup>; <sup>1</sup>Internal Medicine 2, Friedrich-Alexander University, Erlangen, <sup>2</sup>Center of Geriatrics at the University of Heidelberg, Bethanien Hospital, <sup>3</sup>Institute for Innovative Health Management (IIGM), Berlin, <sup>4</sup>Institute for Mental Health-ZI, Mannheim, Germany.

**Introduction:** Up to 2008 there were no evaluated diabetes education programs for geriatric nurses available in Germany. Therefore, the Working group of the German Diabetes Association "Diabetes and Geriatrics" in cooperation with an institute for health management, set up the 10-day diabetes education program for geriatric nurses (DPFK) based on a written curriculum.

**Methods:** The program is split in 10 education days (every 2 weeks). The program focuses on the field of diabetes, nursing and quality management. Education units are f.ex. pathophysiology of diabetes, self-monitoring, diabetes therapy, techniques of insulin injection, diabetes complications, guidance of patients to learn autonomous insulin injection. The aim was to evaluate the effect of the standardized education program for geriatric nurses on their diabetes knowledge using standardized multiple choice questionnaires before (t1), during (t2), at the end (t3) and 3 months after (t4) participation in the education program. High scores in the multiple choice tests reflect better diabetes knowledge of the geriatric nurses (maximum score 75 points, Cronbachs alpha 0.82, mean item difficulty 0.65 T1). The present trial was a prospective randomised trial with one intervention group and two control groups (Internal control group: geriatric nurses working in the same nursing home/ambulatory care as the intervention group participant. External control group: geriatric nurses working in another nursing home/ambulatory care as the intervention group participant).

**Results:** Overall 81 geriatric nurses completed the study (48 intervention group participants, 28 internal control group participants and 15 external control group participants). There were no differences in diabetes knowledge comparing baseline skills of intervention group, internal and external control group participants (see Table 1). Intervention group participants improved their diabetes knowledge significantly. Interestingly, geriatric nurses out of the internal control group improved their diabetes knowledge from T1 to

T4 too, while participants of the external control group did not. These results have been expected, as firstly the concept of the structured education program for geriatric nurses was not only to improve diabetes knowledge of participants but also to change structure and process quality within the nursing homes/ ambulatory care services itself.

**Conclusion:** To summarize, participation in the structured education program was effective in improving diabetes knowledge in geriatric nurses and to change process and structure quality in nursing homes/ ambulatory care services significantly.

Results in diabetes knowledge (points in multiple choice test  $\pm$  SD) of the geriatric nurses

	T1	T2	T3	T4	p-value T1 vs. T4
Intervention group participants (n=48)	27.9 $\pm$ 6.9	39.4 $\pm$ 8.0	58.1 $\pm$ 8.8	59.3 $\pm$ 9.0	<0.001
Internal control group (n=28)	27.1 $\pm$ 9.4	34.8 $\pm$ 12.8	36.8 $\pm$ 14.7	44.0 $\pm$ 17.1	<0.001
External control group (n=15)	30.9 $\pm$ 8.9	30.8 $\pm$ 8.8	35.0 $\pm$ 13.2	33.7 $\pm$ 14.5	0.39
p- values intervention vs. internal control group	0.69	0.1	<0.001	<0.001	-
p-values intervention vs. external control group	0.19	0.001	<0.001	<0.001	-
p- values internal vs. external control group	0.21	0.29	0.70	0.06	-

Supported by: Robert Bosch Foundation Stuttgart, Research Fellowship

## PS 083 Diabetes care in different settings

998

### Use of contraceptives and glucose control in women with type 1 diabetes mellitus

C. Kellner, G. Wolf, U.A. Müller;  
Universitätsklinikum Jena, Germany.

**Background and aims:** Some women with type 1 diabetes mellitus experience worsening of metabolic control before the menstrual period, with a higher appearance of hypo- and hyperglycaemia. Indeed, patch-clamp-analysis has shown an influence of sex hormone fluctuation of glycaemic control. In a detailed German diabetes teaching and treatment program, women with type 1 diabetes are warned about the higher risk of ketoacidosis and hypoglycaemia before menstrual period. They also cover about the possible benefit from using contraceptives to stabilise blood glucose before menstruation. Therefore this study was designed to investigate the association of using contraceptives with lower blood glucose and less acute complications.

**Materials and methods:** Included were women with type 1 diabetes mellitus, aged between 15-50 years, a minimum duration of diabetes of 6 months and a visit in an university hospital (in- and outpatients) between 01.01.1990-01.02.2011. 6.255 visits of patients meet the inclusion criteria. Excluded were all visits without an HbA<sub>1c</sub>-value, a missing medical history, pregnant women, women in lactation and unclear diabetes-type. After exclusion criteria 3.732 visits could be analysed. The data were obtained from the electronic patient record form EMIL \*. The HbA<sub>1c</sub> value was DCCT adjusted (DCCT mean normal range: 5.05%). Use of contraceptives included injectable or oral contraceptives, hormone containing intrauterine device, hormonal contraceptive vaginal ring and contraceptive patch. Severe hypoglycaemia defined as glucagon injection or glucose i.v. was reported as episodes per 12 months. Ketoacidosis is defined as an acute hyperglycaemia with acidosis, which led to hospitalisation.

**Results:** 3321 visits from patients had no use of contraceptives, 411 visits with contraceptives could be identified. Patients with contraceptives were younger (32 vs. 36y,  $p < 0.001$ ), had a lower duration of diabetes (15 vs. 16y,  $p < 0.001$ ), fewer severe hypoglycaemia (0.11 vs. 0.15 episodes per year,  $p < 0.001$ ) and no ketoacidosis (0 vs. 0.04 episodes per year,  $p < 0.001$ ). In a regression analysis with HbA<sub>1c</sub> as a fixed variable and the independent variables age, duration of diabetes, use of contraceptives and number of blood glucose self-monitoring per / week, 2% of the variance in HbA<sub>1c</sub> was determined by age ( $p = 0.001$ , positive association) and diabetes duration ( $p = 0.03$ ; positive association). Use of contraceptives and blood glucose control had no significant association of HbA<sub>1c</sub> in the regression analysis. With regard to severe hypoglycaemia showed no significant associations with age, diabetes duration, blood glucose self-monitoring per week, use of contraceptives or the HbA<sub>1c</sub> value. Four percent of the variance of ketoacidosis has been declared in the regression analysis by a high HbA<sub>1c</sub> ( $p = 0.001$ , positive association). No significant association remained blood glucose self-monitoring per week, duration of diabetes, contraceptive use and age.

**Conclusion:** The use of contraceptives was not related to HbA<sub>1c</sub> or acute complications such as ketoacidosis or severe hypoglycaemia.

999

### Young adult diabetes care in the west of Ireland: challenges and opportunities

R. Casey<sup>1</sup>, M. O Hara<sup>1</sup>, A. Cunningham<sup>1</sup>, M. Bell<sup>1</sup>, H. Burke<sup>1</sup>, D. Wall<sup>2</sup>, R. Geoghegan<sup>3</sup>, L. Hynes<sup>4</sup>, B. McGuire<sup>4</sup>, M. Gately<sup>1</sup>, S.F. Dinneen<sup>1,5</sup>;  
<sup>1</sup>Endocrinology and Diabetes Centre, Galway University Hospital, <sup>2</sup>School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, <sup>3</sup>Paediatrics, Galway University Hospital, <sup>4</sup>School of Psychology, National University of Ireland, <sup>5</sup>School of Medicine, National University of Ireland, Galway, Ireland.

**Background and aims:** The management of young adults with type 1 diabetes poses a number of challenges for both healthcare workers and healthcare organisations. Despite the establishment of a dedicated Young Adult Diabetes Clinic at our University Hospitals in 2005, healthcare professionals do not feel they are impacting on attendance rates and diabetes outcomes among this group and hence an audit of the service was conducted using

the Department's clinical information system and Emergency Department (ED) records.

**Materials and methods:** The reported data are expressed as percentages, means ( $\pm$  standard deviation, SD) unless other stated as the median and range. One-way ANOVA, two-sample t-tests, chi-square tests and Spearman and Pearson's correlations were used to determine association and comparisons between groups.

**Results:** Patients with type 1 diabetes referred to our service aged between 18–25 years before October 2011 were included in this study. The mean ( $\pm$  SD) age of the group ( $n = 137$ ) was 22 years ( $\pm 1.96$ ), 51.6% were male, 45.3 % were living with type 1 diabetes for at least 10 years. The average of all HbA<sub>1c</sub> measurements over 24 months was 81mmol/mol (9.6%). Diabetes complications were documented in 32.1% with retinopathy occurring in 19%. On average these young people were offered 7 clinic appointments in a 24 month period but on average only attended 4 (SD = 3.1). If a patient had not attended a clinic in over two years they were listed as a 'defaulter', 12.4% met these requirements. Unfortunately, 1 of the young adults died during the audit period. Patients who frequently missed clinics had more nurse contacts ( $p < 0.01$ ). ED admissions for both Diabetic Ketoacidosis (DKA, excluding those newly diagnosed) and hypoglycaemia were analysed in a subgroup of patients ( $n = 65$ ) who live within 80km of the base hospital. A total of 74% attended the ED at least once, 46% had 2 or more attendances and 26% had 3 or more. The average length of stay for patients admitted with DKA or severe hypoglycaemia was 2 days. DKA was the cause for ED attendance in 55.4%, 4.6% attended due to a hypoglycaemic event and 13.8% attended with separate hypoglycaemia and DKA episodes. There was no relationship between ED attendance and complication rates ( $p = 0.56$ ), missed clinic appointments ( $p = 0.33$ ) or HbA<sub>1c</sub> ( $p = 0.2$ ). Longer duration of illness was associated with more frequent ED attendances for hypoglycaemia ( $p < 0.01$ ).

**Conclusion:** Our results highlight the challenging issues posed by current healthcare utilisation in this patient group and the extra workload created through missed clinic appointments. We believe a different approach is required emphasising an organisational change that adapts to patient behaviour in young adults with type 1 diabetes.

## 1000

### A brief hospitalisation as an opportunity to improve diabetes control

Y. Bar-Dayán<sup>1</sup>, M. Boaz<sup>2</sup>, T. Chaïmy<sup>3</sup>, Z. Matas<sup>3</sup>, Z. Landau<sup>1</sup>, J. Wainstein<sup>1</sup>;

<sup>1</sup>Diabetes Unit, <sup>2</sup>Epidemiology and Research Unit, <sup>3</sup>Laboratory Unit, Wolfson Medical Center, Holon, Israel.

**Background and aims:** Poorly controlled hyperglycemia is associated with increased morbidity and mortality in hospitalized patients. Based on the view that hospitalization provides a window of opportunity to improve patient care and health status, a comprehensive program for treating hospitalized diabetic patients (PTHDP) was initiated. This study assessed the effectiveness of the PTHDP program.

**Materials and methods:** Pre-test post-test design. In the pre-intervention period (August–December 2007), as the first step of HDP an institution-wide blood glucose monitoring system was introduced in August. The remaining program components were introduced in January 2008, including implementing a hospital care protocol based on the 2007 American Diabetes Association Standards. PTHDP relies on a multidisciplinary team, which participates in patient care and arranges continuing care following discharge. Central to PTHDP was measuring hemoglobin A1C and albuminuria, and comprehensive patient education prior to discharge. The study ran from January 2008 through–October 2011.

**Results:** During follow-up, more than 600,000 blood glucose tests were performed with a mean value of  $186.2 \pm 89.7$  mg/dL. Glucose values declined from  $196.4 \pm 98.4$  mg/dL pre-PTHDP (August–December 2007) to  $174.5 \pm 82.0$  mg/dL post-PTHDP (January–October 2011) ( $p < 0.0001$ ). A significant, inverse association between blood glucose values and time was detected, indicating a reduction in mean blood glucose levels over time in hospitalized patients, concomitant with the introduction of the PTHDP. Blood glucose values declined over time after controlling for age, sex, and type of department. Prevalence of glucose values lower than 60 mg/dL declined from 2% to 1.3% ( $p < 0.004$ ). Prevalence of glucose values  $\geq 300$  mg/dL declined from 13.6% to 8.4% ( $p < 0.0001$ ). Concomitantly, a significant increase in the proportion of in-target values of 80–180 mg/dL was observed, increased from 47.7% to 58.1% ( $p < 0.0001$ ). Comparing the internal medicine to the surgical departments (excluding intensive care units) mean blood glucose values were significantly higher in internal medicine departments  $189.1 \pm 92.0$  mg/dL compared to  $177.3 \pm 79.4$  mg/dL ( $p < 0.0001$ ). Significantly more hypogly-

cemic events occurred in internal medicine departments 1.5% versus 0.7% ( $p < 0.0001$ ), and being in an internal medicine department increased the risk of hypoglycemic events by factor of 2. Hyperglycemic events were more common in internal medicine departments 11.5% versus 7.7% ( $p < 0.0001$ ); and being in an internal medicine department increased the risk by 57%. A significantly greater proportion of blood glucose values were within target values in surgical units 58.6% vs. 51.4%, respectively ( $p < 0.0001$ ). Blood glucose values, hyperglycemic and hypoglycemic events remained higher in internal medicine compared to surgical departments and in-target blood glucose values remained lower in internal medicine compared to surgical departments after controlling for age.

**Conclusion:** PTHDP improved inpatient glycemic control, which continued over time. More resources should be investigated in order to control diabetes in internal departments. The effect of this improvement on inpatient morbidity and mortality needs further follow-up.

## 1001

### Should we screen all acutely unwell patients for hyperglycaemia?

A prospective study at a medical acute admissions unit in the UK

S. Rokadiya, A. Nihat, M. Kostoula, F. Keane, B. Stroll, J. Patel, P. Pusalkar; Acute Assessment Unit, Watford General Hospital, UK.

**Background and aims:** There are currently no guidelines regarding testing capillary blood glucose or plasma glucose levels in acute medical inpatients, nor is there a national screening programme for the diagnosis of diabetes mellitus (DM) in the UK. Several observational studies have pointed to a strong association between hyperglycaemia and poor clinical outcomes including prolonged length of stay, infection, morbidity and mortality. Additionally, patients with impaired glucose tolerance are at increased risk of cardiovascular complications. We aimed to ascertain whether screening patients for hyperglycaemia during their acute hospital admission could identify patients either with DM or those at risk of developing DM.

**Method:** We prospectively analysed A&E, nursing and medical notes of 662 (338 male and 324 female, age range 16 to 96 years) unselected acute medical patients presenting to the Acute Admissions Unit (AAU) over a two-month period. All these patients were admitted in a non-ICU setting. We checked whether these patients had their capillary blood glucose and/or venous plasma glucose measured during the acute phase of their admission (from admission to 24 or 48 hours). Hyperglycaemia was defined as a blood glucose of  $>7.8$  mmol/L (ADA & AACE Consensus statement on inpatient glycaemic control, 2009).

**Results:** Of 662 patients, 159 were known to have DM (24%) and 503 had no history of DM. 140 patients with DM (89%) had their capillary glucose measured and 77 (55%) also had their plasma glucose measured. 19 patients (11%) with DM did not have their capillary blood glucose checked during the acute phase of their admission. Of the 503 patients with no history of DM, 209 (43%) had their capillary blood glucose measured. 37 (18%) of these had a capillary blood glucose of  $>7.8$  mmol/L and of these, 25 (64%) also had venous plasma glucose measurements. Interestingly, 7 (3.3%) patients with no history of DM had a capillary blood glucose of  $>11.1$  mmol/L and only 4 of these had their plasma glucose tested. Initial diagnoses for these patients were of acute coronary syndrome, pneumonia, cellulitis and drug overdose.

**Conclusion:** Only 43% of patients with no history of DM had their blood glucose checked. 18% of acute medical admissions in our cohort (with no previous history of DM) were found to be hyperglycaemic and 3.3% had a capillary blood glucose of more than 11.1 mmol/L. Follow-up of these patients is essential in order to differentiate between stress hyperglycaemia, impaired glucose tolerance and diabetes. In the absence of any robust guidelines, we suggest screening all acute medical patients with a simple capillary glucose measurement on admission. We also suggest that with no national screening programme for diabetes, emergency medical admissions should be used as a good opportunity to screen for diabetes.



## 1002

### Medication review in elderly patients discharged from hospital substantially decreased drug related problems

J. Hugtenburg<sup>1</sup>, A. Ahmad<sup>1</sup>, J.M. Dekker<sup>2</sup>, P.J. Kostense<sup>1</sup>, G. Nijpels<sup>1</sup>;

<sup>1</sup>Clinical Pharmacy, Vrije Universiteit Amsterdam, <sup>2</sup>Epidemiology and Biostatistics, Vrije Universiteit Amsterdam, Netherlands.

**Background and aims:** Older type 2 diabetes patients are using several drugs together. Drug related problems (DRPs) are common among elderly patients who are discharged from the hospital and using several drugs. Few interventions were reported to decrease DRPs in elderly people discharged from the hospital. We therefore studied the effect of medication review by the community pharmacist on the occurrence of DRPs in elderly persons.

**Materials and methods:** A randomized controlled study involving 340 patients. Community pharmacists were randomized to a control and an intervention group. Intervention pharmacists were instructed to perform a medication review, which include a medication analysis, treatment analysis, patient interview and counseling among patients aged over 60 years, using at least five prescription drugs and discharged from the hospital. Pharmacy technicians interviewed and counseled control and intervention patients in order to identify patient-perceived DRPs at baseline. They were instructed to interview and counsel patients at discharge, after three, six and nine months. DRPs in patients of control and intervention pharmacists were measured at baseline and at 12 months by two clinical pharmacologists.

**Results:** The total number of DRPs were 253 and 437 in the control group and 271 and 689 in the intervention group. The mean number of DRPs identified with the medication analysis decreased in the intervention group from 1.51 to 1.37. In the control group, the number of DRPs increased from 1.58 to 1.62. The mean number of DRPs identified with the patient interview in the intervention group decreased from 3.88 to 2.33. In the control group, the mean number of DRPs increased from 2.73 to 2.80. Subgroup analyses showed that the reduction of DRPs identified with medication analysis was significantly more pronounced among patients with hypertension ( $p = 0.011$ ) and heart failure ( $p = 0.001$ ).

**Conclusion:** The present study showed that pharmacist-led medication review is an effective method to reduce DRPs among elderly patients discharged from the hospital.

*Clinical Trial Registration Number: NTR1036*

*Supported by: ZonMw*

## 1003

### Disparities of diabetes care in rural versus urban communities: more than a comparison

C. Burnot<sup>1</sup>, P. Vidal<sup>2</sup>, J. Lecadet<sup>2</sup>, J. Hazart<sup>2</sup>, S. Maqdasy<sup>1,3</sup>, L. Arnaud-Charra<sup>1</sup>, B. Roche<sup>1</sup>, F. Desbiez<sup>1</sup>, M. Batisse-Lignier<sup>1,3</sup>, I. Tauveron<sup>1,3</sup>;

<sup>1</sup>Departement of Diabetes, CHU, Clermont Ferrand, <sup>2</sup>Assurance-Maladie, Clermont Ferrand, <sup>3</sup>UMR CNRS 6293-Clermont University, INSERM U 1103, France.

**Background and aims:** The aim of our study is to compare the management of patients with type 2 diabetes in the Puy de Dome, area in France, to assess if there is any difference in terms of follow-up recommended by the High Authority for Health in patients living in rural communities versus urban ones.

**Materials and methods:** This is a retrospective case - control study. Inclusion criteria were: patients older than 50 years old, affiliated to the national health insurance, living in Puy de Dome, receiving at least one oral anti-diabetic medication or insulin repaid from April 1, 2010 to March 31, 2011. Rural population was defined by an access time to a hospital more than 1 hour away. Patients were matched for age and for each rural patient, 2 urban were included. The studied variables were HbA1c assays, fasting plasma glucose, lipid determinations, microalbuminuria, serum creatinine levels, ophthalmoscopy, cardiac evaluation and dental care. The number of general practitioner or diabetes consultations and hospitalizations were evaluated.

**Results:** Overall, 1089 rural patients and 2178 urban patients were included. Mean age was 68,0 years  $\pm$  9.8 and sex ratio was 1:4. 20.5% of rural patients versus 14.7% of urban patients did not have HbA1c any early evaluation ( $p < 0.01$ ). Among those who had an HbA1c, the average annual number of HbA1c evaluations was 1.6 for those living in rural areas and 1.7 for those living in urban areas ( $p < 0.01$ ). Furthermore, annual assays are significantly lower in rural areas vs urban zones concerning micro albuminuria (20.8% vs 29.1% respectively;  $p < 0.01$ ) fasting plasma glucose (64.2% vs 70.7%;  $p < 0.01$ ) and lipid assays (68.1% vs 75.7%;  $p < 0.01$ ) but no difference was noticed for

creatinine sampling (83.3 % vs 81.2 %). Moreover, medical follow up is significantly lower in rural areas: ophthalmologic screening (43.2% vs 49.8%;  $p < 0.01$ ) cardiac evaluation (36.8 vs 45.3%;  $p < 0.01$ ), dental care (28.3% vs 38.8%;  $p < 0.01$ ), general practitioner follow up (7.9 vs 8.3 consultations /year;  $p < 0.02$ ) or diabetes consultations (8.4% vs 13.2%;  $p < 0.01$ ). Further, all combined hospitalizations are significantly lower in rural zones (28.9% vs 31.1%;  $p < 0.02$ ). Sub-analysis of these parameters by gender and by age groups confirmed these results.

**Conclusion:** The care of diabetic patients in rural areas is lower than patients living in urban areas. Many factors can explain these discrepancies among which: a more difficult access to care and educational programs and lower income. These differences could still be higher with the spread of desertification of medical care in isolated areas. Thus, public health policies should focus on reducing geographical inequalities by promote medical proximity.

## 1004

Withdrawn

## 1005

### Development and validation of a clinician-administered assessment tool that predicts self-efficacy in MDI management

I. Cranston<sup>1</sup>, J. Ryder<sup>2</sup>, C. Vogel<sup>3</sup>, C.G. Parkin<sup>4</sup>, D.A. Cavan<sup>2</sup>, R. Ziegler<sup>5</sup>, K. Barnard<sup>6</sup>, W. Koehler<sup>7</sup>, I. Vesper<sup>8</sup>, B. Petersen<sup>8</sup>, M. Schweitzer<sup>8</sup>;

<sup>1</sup>Queen Alexandra Hospital, Portsmouth, UK, <sup>2</sup>Royal Bournemouth Hospital, UK, <sup>3</sup>Internistisches Facharztzentrum, Langen, Germany, <sup>4</sup>CGParkin Communications, Inc., Boulder City, USA, <sup>5</sup>Diabetes Clinic for Children and Adolescents, Muenster, Germany, <sup>6</sup>University of Southampton, UK, <sup>7</sup>baseline statistics GmbH, Mannheim, Germany, <sup>8</sup>Roche Diagnostics GmbH, Mannheim, Germany.

**Background and aims:** Safe and effective diabetes therapy with multiple daily insulin injections (MDI) requires that patients possess both knowledge and confidence in their ability to count carbohydrates (CHO) and calculate bolus insulin doses. We developed a short, simple-to-administer questionnaire to assess baseline awareness of the skills required to effectively manage MDI therapy. We administered the tool to patients with type 1 (T1DM) and type 2 (T2DM) to evaluate its ability to predict self-efficacy in MDI management and to identify any correlations with glycaemic status.

**Materials and methods:** Baseline data were drawn from 93 T1DM and T2DM subjects enrolled in the Automated Bolus Advisor Control and Usability Study (ABACUS), a large, prospective, randomized, multi-national study of poorly controlled ( $\text{HbA1c} \geq 7.5\%$  / 58 mmol/l) MDI-treated patients. Characteristics were: mean (SD) age 41.1 (13.4) years, male 54.8%, diabetes duration 16.4 (10.5) years, MDI treatment duration 10.5 (7.7) years, and time since CHO training 40.1 (32.9) weeks. Subject history of MDI skills training was not captured. At screening, investigators administered the tool during a semi-structured interview to assess both carbohydrate counting skills and insulin dose adjustment skills. Tool scores were divided into 2 sub-categories: CHO assessment; and MDI assessment.

**Results:** Within this cohort, 54 (58.1%) subjects scored full competence (perfect score) in all questions in the CHO subset; whereas, only 26 (28.3%) achieved a perfect score in the MDI subset. Gender-related differences in scores were seen, with females showing a higher CHO-related perfect score (71.4% vs. 47.1%,  $p < 0.05$ ) but a slightly lower MDI-related perfect score (19.5% vs. 53.3%,  $p = \text{ns}$ ). A perfect score in either the CHO or MDI subset was associated with a lower mean (SD) baseline HbA1c: 8.57% (0.88) vs. 9.21% (1.55),  $p < 0.05$ ; and 8.32% (0.82) vs. 9.06% (1.33),  $p < 0.05$ , respectively. Subjects with perfect scores in both subsets ( $n=19$ ) had lower HbA1c values than those scoring perfectly in either one ( $n=35$ ) or no subset ( $n=39$ ): 8.24% (0.51) vs. 8.77% (1.0) vs. 9.21% (1.55), all  $p < 0.05$ . Better MDI assessment was associated with fewer blood glucose levels above 300 mg/dl ( $r = -0.34$ ).

**Conclusion:** These findings suggest that use of this simple questionnaire can predict self-efficacy and glycaemic outcomes in T1DM and T2DM patients treated with MDI. The differences seen in "fully competent" subjects suggest a level of self-management competence, which is associated with improved glycaemic outcomes that are similar to those seen with therapy change, such as commencement of CSII.

*Clinical Trial Registration Number: NCT01460446*

*Supported by: Roche Diagnostics, Inc.*

## PS 084 Learning from the global pandemic

### 1006

#### Boehringer Ingelheim employee study: a prospective epidemiological cohort study diabetes prevalence and association between impaired glucose metabolism and risk factors

M. Schneider<sup>1,2</sup>, S. Martin<sup>3</sup>, R.D. Hilgers<sup>4</sup>, K. Dugi<sup>5</sup>, C.W.v. Wolmar<sup>6</sup>, B. Haastert<sup>7</sup>, K. Kempf<sup>8</sup>;

<sup>1</sup>Medical Corporate Department, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, <sup>2</sup>Mannheim Institute for Public Health, Medical Faculty Mannheim, <sup>3</sup>West-German Center of Diabetes and Health, Düsseldorf Catholic Hospital Group, <sup>4</sup>Department of Medical Statistics, RWTH-Aachen University, <sup>5</sup>Boehringer Ingelheim Pharma GmbH, Ingelheim, <sup>6</sup>Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany, <sup>7</sup>mediStatistica, Neuenrade, Germany.

**Background and aims:** Epidemiologic studies in Germany have shown that in the age-group of 55-74 years old the prevalence of type 2 diabetes mellitus (T2DM) ranges about 16% and that glucose metabolism is disturbed in 40%. Since this problem not only has a health-economic impact but also impairs companies, we investigated by the Boehringer Ingelheim Employee Study the prevalence of T2DM in a representative cohort of the working population and associations between impaired glucose metabolism and cardiometabolic risk factors.

**Materials and methods:** Employees (>40 years; n=4326) of Boehringer Ingelheim Pharma GmbH & Co.KG were invited by the medical corporate department to participate in the prevention and health care program 'FIT IM LEBEN - FIT IM JOB'. The program offers every 3-5 years intensive health check-ups for free and advices for lifestyle changes. Cross-sectional analysis of baseline data was performed in order to describe the prevalence of T2DM (self-reported and/or HbA1c >6.5% and/or fasting glucose >126 mg/dl), hyperinsulinemia (fasting insulin >15 µU/ml), insulin resistance (HOMA index ≥2.6) and metabolic syndrome (according to the definition of the International Diabetes Federation (IDF)). Associations had been analyzed by Pearson correlation and differences between subgroups had been determined using t-test and odds ratios (OR) with 95% confidence intervals (CI).

**Results:** 90% of employees (n=3907; 54% men, mean age 47 ± 6 years) joined the programme. T2DM was prevalent in 15%, hyperinsulinemia in 12% and insulin resistance in 23%. Fasting insulin (r=0.396 and r=0.387; respectively, p=0.0001 each) were significantly correlated with body mass index and waist circumference. In persons with the metabolic syndrome or HbA1c above 6.5% fasting insulin and HOMA index were significantly elevated (p<0.0001 each) compared to the remaining cohort. In case of insulin resistance the risk for metabolic syndrome (OR 7.8 [6.0; 10.0]), hypertension (OR 3.0 [2.4; 3.9]), hypertriglyceridemia (OR 4.0 [3.2; 5.0]), hypercholesterolemia (OR 1.3 [1.1; 1.7]), increased intima media thickness (OR 2.6 [1.4; 4.7]) and cardiovascular diseases (OR 1.3 [1.0; 1.7]) was significantly increased.

**Conclusion:** Impairments in glucose and insulin metabolism and their associations with cardiometabolic risk factors are serious health problems in the working population. Since serious inducements for prevention programs by health insurances are lacking, companies should investigate in health care programs for their employees to reduce incidental wage costs. Due to their representative and high amount of participants and their intensive phenotyping the Boehringer Ingelheim Employee Study allows analyzing a variety of occupational health care questions.

*Supported by: Boehringer Ingelheim*

### 1007

#### Boehringer Ingelheim employee study: a prospective epidemiological cohort study Prevalence of and association between cardiometabolic risk factors and diseases

K. Kempf<sup>1</sup>, S. Martin<sup>1</sup>, R.D. Hilgers<sup>2</sup>, K. Dugi<sup>3</sup>, C.W.v. Wolmar<sup>4</sup>, B. Haastert<sup>5</sup>, M. Schneider<sup>6,7</sup>;

<sup>1</sup>West-German Center of Diabetes and Health, Düsseldorf Catholic Hospital Group, <sup>2</sup>Department of Medical Statistics, RWTH-Aachen University, <sup>3</sup>Boehringer Ingelheim Pharma GmbH, Ingelheim, <sup>4</sup>Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, <sup>5</sup>mediStatistica, Neuenrade, <sup>6</sup>Medical Corporate Department, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, <sup>7</sup>Mannheim Institute for Public Health, Medical Faculty Mannheim, Germany.

**Background and aims:** Prevalence of overweight, obesity and reduced physical activity increased in recent years and supports the development of hypertension, dyslipidemia and type 2 diabetes mellitus, which are the main risk factors for cardiovascular diseases. Myocardial infarction and apoplexy not only impair quality of life and elevate the costs for the health care system but also burden companies by increasing costs for non-productive time and early retirement. However, valid and representative data about prevalence of cardiovascular risk factors in operational medicine are lacking so far.

**Materials and methods:** Employees (>40 years; n=4326) of Boehringer Ingelheim Pharma GmbH & Co. KG were invited by the medical corporate department to participate in the prevention and health care programme 'FIT IM LEBEN - FIT IM JOB'. The program offers every 3-5 years intensive health checkups for free and advices for lifestyle changes. Cross-sectional analysis of baseline data was performed in order to describe the prevalence of cardiometabolic risk factors and diseases. Differences between subgroups had been determined using t-test and odds ratios (OR) with 95% confidence intervals (CI).

**Results:** 90% of employees (n=3907; 54% men, mean age 47 ± 6 years) joined the programme. 40% of participants were overweight (body mass index (BMI) 25-30 kg/m<sup>2</sup>) and 16% obese (BMI >30kg/m<sup>2</sup>). Fitness was below standard (= lower tertile) in 31%. Prevalence of cardiometabolic risk factors and diseases such as hypercholesterolemia was 61%, hypertension 35%, metabolic syndrome (according to the definition of the International Diabetes Federation (IDF)) 27%, hypertriglyceridemia 23%, insulin resistance 23%, diabetes mellitus 15%, hyperinsulinemia 12%, increased intima media thickness 11% and cardiovascular diseases (e.g. myocardial infarction, apoplexy) 16%. Overweight and obese subjects had significant higher waist circumference, HbA1c, blood pressure, triglycerides, total and LDL cholesterol, intima media thickness and lower HDL cholesterol (all p<0.0001). Prevalence of diabetes mellitus significantly increased the risk for plaques within the Aorta abdominalis (OR 1.7 and CI [1.2; 2.4]), within the Aorta carotis (OR 3.4 [2.5; 4.6]) or stenoses therein (OR 8.3 [3.1; 22.5]).

**Conclusion:** The Boehringer Ingelheim Employee study demonstrates that the prevalence of cardiometabolic risk factors and diseases is a serious problem in the group of employees. Since risk factors can be reduced by lifestyle changes and by offering prevention programs, companies have the opportunity not only to increase the quality of life of their employees but also to reduce incidental wage costs.

*Supported by: Boehringer Ingelheim*

### 1008

#### The optimal cut-off value of waist circumference based on insulin resistance and visceral fat thickness in Korean type 2 diabetes

J. Lim<sup>1</sup>, Y. Choi<sup>2</sup>, E. Lee<sup>1</sup>, K. Huh<sup>2</sup>;

<sup>1</sup>Yonsei University College of Medicine, <sup>2</sup>Huh's Diabetes Center and the 21 Century Diabetes and Vascular Research Institute, Seoul, Republic of Korea.

**Background and aims:** Central obesity is an important contributor to the development of metabolic syndrome and cardiovascular diseases (CVDs). In addition, visceral fat thickness (VFT) is known to be a reliable index for detecting diabetic patients who are at high risk of CVDs. However, there was an paucity of data on the appropriate cutoff point of waist circumference (WC) associated with metabolic risk factors, mainly targeted at type 2 diabetes. The aim of this study is to investigate the optimal cutoff value of WC for visceral obesity in type 2 diabetes.

**Materials and methods:** We evaluated 6,579 diabetic patients (male 3,394, female 3,185, mean age 58.03 ± 10.64 yr) who visited our Diabetes Center from Jan 2003 to Jun 2009. WC was measured at the midpoint between the lower

rib and the iliac crest, and insulin sensitivity was assessed by a rate constant for plasma glucose disappearance (*Kitt* %/min) using insulin tolerance test. The presence of visceral obesity, which was measured by ultrasonography, was defined as VFT of more than 47.6 mm and 35.5 mm in men and women, respectively, according to the criteria for diabetic patients as described by Kim et al. Statistic analysis was performed using ROC curve.

**Results:** The optimal cutoff point of WC for identifying two or more metabolic components was 87 cm for men and 81 cm for women. Moreover, these cutoff points had a higher association with the degree of visceral obesity in subjects with insulin resistance (the lowest tertile of *Kitt*) than insulin sensitive subjects (the highest tertile of *Kitt*).

**Conclusion:** Our results suggest that the optimal cutoff values of WC for insulin resistance and visceral obesity in Korean type 2 diabetes should be reestablished.

## 1009

### Long term projection of diabetes-related complications and life expectancy in China

G. Ning<sup>1</sup>, L. Wang<sup>2</sup>, Y. Jiang<sup>2</sup>, Y. Li<sup>2</sup>, N. Lund<sup>3</sup>, F. Sun<sup>4</sup>, Y.-F. Bi<sup>1</sup>, Y. Xu<sup>1</sup>, L. Wang<sup>2</sup>;

<sup>1</sup>Department of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai, <sup>2</sup>National Center for Chronic and Noncommunicable Disease Control and Prevention, Beijing, <sup>3</sup>Novo Nordisk, Copenhagen, Denmark, <sup>4</sup>Novo Nordisk China Pharmaceuticals Ltd, Beijing, China.

**Background and aims:** The Chinese government has a target of increasing life expectancy by one year by 2015 with the completion of the Twelfth Five-Year-Plan for national health development of the People's Republic of China. The China Non-communicable Chronic Disease (NCD) Surveillance revealed high prevalence of diabetes in China (9.7%) equal to 93 million people. IDF projects that this number will rise to 129.7 million in 2030. This increase in the number of people living with diabetes can put the life expectancy improvement target at risk. The burden on the Chinese healthcare system is expected to be significant, if efforts to contain the disease are not made. We attempt to estimate what these effects could be of limited additional intervention compared to meeting glycaemic targets at different level of success.

**Materials and methods:** For the analysis, the CORE Diabetes Model with China-specific modifications was used. The patient cohort used was based on the diabetes sub-population of the China NCD Surveillance supplemented with data from the observational studies A1chieve and DiabCare where data from the NCD Surveillance was not available. In addition to measures of glycaemic control, the clinical inputs were as follows (mean (sd)): SBP 144 mmHg (25.1), TChol 4.5 mmol/L (1.3), HDL 1.06 mmol/L (0.32), LDL 2.57 mmol/L (0.87), Trigs 2.09 mmol/L (2.27), BMI 25.3 kg/m<sup>2</sup> (3.83) and 27.6% smokers. Mean age was 54.4 years. 63.9% were newly diagnosed. 56% were male. The analysis was based on a closed cohort with no dynamic effects of increased incidence. The modelling time horizon was 25 years and a discounting rate of 3% was used on both clinical and economic parameters.

**Results:** Achieving a glycaemic target of HbA1c < 7.0% for half the population leads to an improvement in life expectancy of around half a year for both gender. Significant improvements, particularly for micro-vascular complications are identified. Given the relatively young age of the cohort and the large share of newly diagnosed there are still benefits of improved treatments beyond the modelling time horizon, as 22% and 30% of men and women respectively were alive at the end of the 25 years in the baseline analysis with 3–5% percentage points more in the improved treatment scenarios. Further reductions are seen if 75% of the population reach the treatment target. Detailed results are shown in the table.

**Conclusion:** Even moderate improvements in glycaemic control can reduce diabetes related complications - particular micro-vascular complications - increase life expectancy and improve quality of life for people living with diabetes in China.

	Male No additional treatment	Male 50% to HbA1c<7.0%	Male 75% to HbA1c<7.0%	Female No additional treatment	Female 50% to HbA1c<7.0%	Female 75% to HbA1c<7.0%
Life expectancy (years)	10.9	11.3	11.4	12.1	12.4	12.6
Undiscounted Life expectancy (years)	14.5	15.0	15.3	16.3	16.8	17.1
Quality-Adjusted Life expectancy (years)	7.4	7.7	7.8	8.2	8.5	8.7
Undiscounted Quality-Adjusted Life expectancy (years)	9.7	10.1	10.4	10.9	11.4	11.7
<b>Cumulative incidence of complications</b>						
Background diabetic retinopathy	17.8	11.1	8.8	19.5	12.5	10.0
Severe Vision Loss	9.6	7.1	6.1	10.8	8.2	7.1
Cataract	9.0	7.4	6.7	9.9	8.3	7.5
End stage renal disease	3.2	1.3	1.0	3.4	1.6	1.1
Ulcer	36.1	33.2	32.0	39.4	36.5	35.0
Neuropathy	48.8	36.5	30.8	54.1	41.1	34.6
CHF, death	9.9	9.0	8.4	16.2	14.5	13.7
CHF, event	20.5	17.6	16.3	29.3	25.6	24.0
Angina	18.6	16.9	16.2	14.0	12.4	11.9
Stroke death	12.3	11.4	11.2	12.2	11.4	11.2
Stroke event	23.5	21.6	21.0	22.8	21.1	20.5
MI, death	23.0	22.9	22.2	12.3	12.2	11.5
MI, event	26.4	25.9	24.5	16.9	16.6	15.6

## 1010

### Physical activity patterns and their metabolic influence in Transylvanian, Romanian type 2 diabetic patients

M.I. Szabo<sup>1,2</sup>, R. Balogh<sup>2</sup>;

<sup>1</sup>Diabetes, Medical and Pharmaceutical University, <sup>2</sup>Diabetes, Emergency County Clinic, Tg Mures, Romania.

**Background and aims:** The beneficial effect of physical activity in type 2 diabetes, and in preventing cardiovascular morbidity is very well established, and exercise guidelines for that category of patients widely exist, however the implementation of this guidelines among diabetic patients is very low, and varies with different habits and barriers in different regions. Our goal was to establish the exercise patterns of type 2 diabetes patients in our region (after our knowledge no such study has so far been done), and their influence on the metabolic outcomes, in order to be able to develop a specific exercise strategy for our patients.

**Materials and methods:** The cross sectional observational study was conducted in Tg Mures, a representative Romanian, Transylvanian day care centre, with patients coming urban and rural environment. A group of 412 type 2 diabetes patients were randomly selected. We recorded socio demographic, anthropometric, diabetes related data, presence of complications, and HbA1c values. Habits of exercise were recorded in detail using a questionnaire, referring to duration, intensity, type, frequency of activity. We introduced the notion of mean physical activity index (PAI)/week (taking in account duration and energy cost of activity) to compare and correlate the different exercises. Effort was divided in 4 categories: walking (1), gardening/working on field type activity (2), profession related activity (3) and sports or leisure physical exercise (4).

**Results:** Women represented 54.3% of the study population; mean age was 63.17±10.05 years. Only 13.6% of patients had an activity level equivalent or greater to that recommended in guidelines (150 min moderate exercise/week). The distribution of activity type chosen by patients was walking: 55.1%, gardening: 31.3%, professional activities: 10.3%, sports only in 3.3% of cases. But the PAI/week was the highest in the 2. and 3. group (p<0.001, CI 0.45-0.25). Men, rural habitants, and those with lower educational level (p<0.01) had higher PAI. The index was inversely correlated with age, BMI (r = -.202, resp -.208, p<0.001). Only the highest PAI quartile had significantly lower HbA1c levels (7.28 vs. 7.92, p<0.01). The PAI was lower in those with cardiovascular comorbidities (p<0.001), but was independent from micro-vascular complications.

**Conclusion:** Despite of exercise recommendations only a small proportion of patients are achieving the goal, compliance is far better with domestic or professional activities than with sports; women, patients with higher educational level, and those living in town are less active. Patterns of activity in our region are different, and there is the need to develop specific, individualised exercise strategies for our patients.



## 1011

**Correlates of physical fitness in patients with type 2 diabetes**J. Puder<sup>1</sup>, L. Allet<sup>2</sup>, F. Amati<sup>3</sup>, J. Barral<sup>3</sup>, P.-M. Marques-Vidal<sup>1</sup>, O. Giet<sup>1</sup>;<sup>1</sup>CHUV, Lausanne, <sup>2</sup>HUG, Genève, <sup>3</sup>UNIL, Lausanne, Switzerland.

**Background and aims:** Several fitness outcomes such as muscular strength, aerobic fitness or walking speed are important predictors of mortality and morbidity. We tested if different physical fitness measures correlated to classical morbidity and mortality outcome measures such as BMI, cardiovascular risk factors, metabolic control, diabetic complications and socioeconomic status in patients with type 2 diabetes.

**Methods:** Seventy-seven patients from 8 DIAfit centers in the French part of Switzerland were recruited to participate in the study. Hypoglycemic episodes, medication use, BMI, blood pressure, vibration threshold (a measure of neuropathy), lipid profile and HbA1c were assessed. Educational level (EL) was used as proxy for socioeconomic status. We tested the following fitness outcomes: Aerobic fitness (maximal cycle ergometry; W), walking speed (required time for 10m walk; sec), functional lower limb muscle strength (required time for chair stand; sec), balance (maximal time for unipedal stand; sec) and flexibility (finger-floor distance; cm).

**Results:** All seventy-seven patients (age  $60.2 \pm 10.3$  yrs (range 30–85 yrs), 58% female, diabetes duration  $9.3 \pm 7.2$  yrs) participated in the study. 6% had at least weekly hypoglycemic episodes. 77% had treated hypertension, 62% had treated dyslipidemia. For 3 patients, falls have been recorded in the last year. The BMI was  $31.8 \pm 4.9$  kg/m<sup>2</sup>, the vibration score  $5.5 \pm 1.9$  and the HbA1c  $7.3 \pm 1.7\%$ . 41% of the participants had a high, 37% a middle and 22% a low EL. Among all 5 fitness measures, age was inversely correlated with aerobic fitness and balance ( $r = -0.3$ ,  $p = 0.02$  and  $-0.4$ ,  $p = 0.001$ ). Furthermore, aerobic fitness was higher in men compared to women ( $p = 0.0001$ ). In this population, BMI, the vibration threshold, HbA1c levels, the presence of hypertension or of dyslipidemia were not associated with physical fitness measures except for a positive correlation between the vibration sensibility and balance ( $r = 0.3$ ,  $p = 0.04$ ). In contrast, EL was associated with aerobic fitness ( $r = 0.48$ ,  $p = 0.001$ ), muscular strength ( $-0.4$ ,  $p = 0.006$ ) and walking speed ( $-0.47$ ,  $p = 0.001$ ).

**Conclusion:** In this population of mostly treated patients with type 2 diabetes, classical morbidity and mortality outcome measures were not associated with different physical fitness measures. In contrast, socioeconomic status was highly associated with physical fitness measures in these patients. These findings provide insight into different modulable clinical pathways with the perspective to further improve outcomes.

Clinical Trials Registration Number: NCT01289587

## 1012

**Burden of disease and out-of-pocket expenditures associated to type 2 diabetes and its complications in Argentina**J.F. Elgart<sup>1</sup>, J.E. Caporale<sup>1</sup>, L. Gonzalez<sup>1</sup>, J.L. De la Fuente<sup>2,3</sup>, M.C. Camillucci<sup>2,3</sup>, J.J. Gagliardino<sup>1</sup>;

<sup>1</sup>CENEXA - Centro de Endocrinología Experimental y Aplicada (UNLP-CONICET La Plata), La Plata, <sup>2</sup>Fundación para las Ciencias Biomédicas de Córdoba, <sup>3</sup>Hospital Privado Centro Médico de Córdoba, Argentina.

**Background and aims:** To assess a) the economic impact of diabetes on quality of life, disabilities and health utilities, on the income, employment, education and property of patients and their families, and b) the efficiency of medical care received by people with type 2 diabetes (T2DM) with and without complications in Argentina; c) to compare these costs with those recorded in people without diabetes.

**Methodology:** Case-control study comparing cases with T2DM with or without chronic micro- and macrovascular complications and controls without diabetes (paired by age and gender) and social contexts. Data were obtained by telephone interviews (March to April 2011) using the same questionnaire except for a few questions related to diabetes treatment. Interview data were supplemented with data obtained from clinical records from each interviewee to fully describe the subjects' resources utilization and costs of care. Data were statistically analysed using the Statistical Package for Social Sciences (SPSS) version 15. Differences in means and proportions were verified using Student's t-test, ANOVA and Chi2. The study had ethical clearance and all subjects provided oral informed consent.

**Results:** We interviewed 1908 patients (954 non diabetic controls, 477 people with T2DM without complications, and 477 people with T2DM and complications), mostly female (52.4%, 56.6% and 50.5%, respectively). Diabetes duration in the group with complications was significantly higher than in the

group without complications ( $8.3 \pm 8.6$  vs.  $11.1 \pm 9.0$ ). People with T2DM with complications had significantly lower salaries; a lower percentage of people attained university degree and showed higher medical resource consumption. They also lost more working days (14.3) than those with T2DM without complications (8.6) or without T2DM (8.8).

**Conclusion:** This is the first report showing objectively the heavy burden of diabetes and its complications for the patient, his/her family, the health system and society overall in a Latin American country. The data are a serious warning for health care authorities to strengthen the implementation of prevention strategies to reduce this burden.

Main results			
Parameter	Control	T2DM without complications	T2DM with complications
Age (yrs; mean $\pm$ SD)	60.6 $\pm$ 11 (954)	61.2 $\pm$ 11.8 (477)	64.5 $\pm$ 8.7 #* (477)
Monthly household income (ARS; mean $\pm$ SD)	5684 $\pm$ 4012 **† (347)	4742 $\pm$ 3956 (174)	4240 $\pm$ 3548 (206)
Monthly household expenditure (ARS; mean $\pm$ SD)	4243 $\pm$ 2613 (188)	3620 $\pm$ 2377 (120)	3840 $\pm$ 2726 (132)
Education (finished University degree)	29.8% *† (279)	19.3% (91)	16.4% (77)
Use of medical services	73.0% (693)	82.7% # (392)	87.1% # (412)
- Doctor office visits	41.1%	80.8% #	90.2% # *
- Medical practices	24.9%	51.1% #	64.5% # *
- Medication	16.2%	59.8% #	79.2% # *
- Laboratory tests	18.9%	60.1% #	71.3% # *
- Hospitalization	4.0%	4.7%	9.8% #
Have you hired someone to take care of you because of the illness? (yes)	2.8% (26)	1.9% (9)	5.1% # (24)
Does someone from your family take care of you because of illness? (yes)	7.6% (67)	6.5% (30)	18.3% #* (83)
Health Status Scale (EQ-5D)	76.3 $\pm$ 15.7 *† (920)	72.6 $\pm$ 15.7 † (466)	68.8 $\pm$ 18.7 (453)

Significant difference ( $p < 0.05$ ) compared to # "Control", to \* "T2DM without complications" and to † "T2DM with complications". N in brackets.

Supported by: Novo Nordisk

## 1013

**Immediate feedback of HbA<sub>1c</sub> results to patients improves diabetes control and outcomes: a 12-month intervention in 10 sub Saharan African settings**E. Sobngwi<sup>1</sup>, N.M. Balde<sup>2</sup>, A. Camara<sup>2</sup>, J.-L. Nguewa<sup>3</sup>, S. Limen<sup>3</sup>, IA3 investigators;

<sup>1</sup>Institute of Health and Society, Newcastle University, Newcastle upon Tyne, UK, <sup>2</sup>Department of Endocrinology, CHU Donka, Conakry, Guinea,

<sup>3</sup>Department of Endocrinology, Yaounde Central Hospital, Cameroon.

**Background and aims:** The objective was to determine whether the introduction of routine affordable HbA<sub>1c</sub> determination with immediate feedback to patients and relevant education in an underserved population without any further intervention on drug supply would significantly improve diabetes control.

**Materials and methods:** We enrolled consecutive people with diabetes diagnosed at least one year prior to the investigation for a 12-month intervention where each participant is its own control. The study settings were 10 existing diabetes care centre in two countries, including 4 regional centres in Guinea and 6 regional centres in Cameroon. All participants had a baseline assessment of diabetes control and complications, and patient knowledge regarding diabetes and its care, a three-monthly assessment of diabetes control with immediate feedback to patient and provision of targeted education, until completion of the planned 12 months follow up. The intervention consisted of point of care measurement of HbA<sub>1c</sub> with immediate feedback to patient and provision of targeted education, in addition to usual care. Education is provided in the format of a one-to-one 10-minute education session with explanation about what HbA<sub>1c</sub> is, its clinical and prognostic meaning, and translation of measurement into average blood glucose value conducted before the routine follow up consultation.

**Results:** A total of 1349 were enrolled including 813 in Cameroon and 536 in Guinea. The mean age of the study population was  $56.2 \pm 12.6$  years and the mean duration of diabetes was  $7.4 \pm 6.3$  years. The study population was composed of 59.8% women in total. Baseline HbA<sub>1c</sub> was  $9.7 \pm 2.6\%$  in Guinea and  $8.6 \pm 2.5\%$  in Cameroon. The average one-year reduction in HbA<sub>1c</sub> was  $-0.8 \pm 2.3\%$  with 40% of the total sample who reduced their HbA<sub>1c</sub> by more than 1 point and 36% at 12-month versus 25% at baseline achieving the  $<7\%$

HbA1c target. The proportion of patients achieving the target changed from 10–39% at baseline across centres, to 19–56%. In parallel, 38 to 71% of the patients decreased their urinary albumin excretion within one year. There was on average a 0.5 to -12 mmHg decrease in systolic blood pressure across the centres. All reported changes in absolute and relative expression in HbA1c, blood pressure and urinary albumin excretion being significant.

**Conclusion:** Improving access to HbA1c point of care determination with immediate feedback to patient and provision of targeted education without additional intervention resulted in a significant improvement in diabetes control as well as blood pressure and urinary albumin excretion in patients with prior minimum one year regular follow up two sub Saharan African countries. These results suggest that the proposed intervention is potentially markedly cost saving.

*Clinical Trials Registration Number: NCT01460095*

*Supported by: BRIDGES (IDF/LILLY)*

## 1014

### Monitoring of diabetes trials and changes made to trial records through automated semantic web-mining

D. Cocker<sup>1</sup>, J. Lievens<sup>1</sup>, M. De Cré<sup>1</sup>, I. Gallen<sup>2</sup>;

<sup>1</sup>MDCPartners, Aartselaar, Belgium, <sup>2</sup>Wycombe Hospital, High Wycombe, UK.

**Background and aims:** There is a challenge to populate large clinical trials in diabetes. Poor enrolment and patient retention are often cited as major causes of study delay and failure. The obligation to publicly disclose trial information today offers the possibility to monitor trial plans and changes made to ongoing trials. Such data can enhance assumptions and feasibility evaluation of new trials. This paper evaluates the results of a robotic, semantic analysis of registered diabetes trials to determine geographic distribution of trial sites and assess changes to trials over time.

**Materials and methods:** We have developed a code-base that extracts data from multiple public databases pertaining to clinical and medical research. Using a medical ontology and natural language processing, resolution of inadequate registry fields is increased and data are semantically linked into one domain containing trials, researchers, organizations, drugs, and geographical information. We compared data sets from December 2008 and January 2012. Industry-sponsored trials recruiting at least 200 diabetes patients in December 2008 (228 trials) were evaluated for changes to individual trial records. An assessment was made of phase III trials for which 2 sites or more had been added according to the January 2012 registry.

**Results:** Diabetes trials are mainly conducted in North America, Western and Eastern Europe. Over the last 5 years, the share of sites opened in these “traditional” diabetes trial regions has remained stable (45, 19 and 10% of all diabetes sites opened), notwithstanding an increase in sites opened in other regions. Number of sites has tripled in East Asia and doubled in Japan (to reach 7.3% and 6.2% of all sites opened in 2011). The percentage of trial sites has also tripled in the Middle East (to 2.1% of all sites opened in 2011). In India, South-East Asia and Russia there has been a marked relative decrease (of at least 50%) in diabetes trial sites opened over the last 5 years. Assessment of changes made to a cohort of industry-sponsored trials shows that about 80% of these trials (186 out of 228) deviate from the initial stated targets for enrolment, study end or number of sites used. Most frequently, enrolment targets are increased (45% of trials increase enrolment by at least 5 patients) or study end date is delayed (in 41% of diabetes trials study end date is delayed by at least 3 months). In 20% of trial records, the site number is increased by at least 2 while the trial is running. When site number is increased, this can indicate an unexpected increase in enrolment need. A cohort of industry-sponsored trials was selected for which site number was increased between 2008 and 2012 and geographic distribution of the newly opened sites was assessed. While the new trial sites were spread over 73 different countries, there was a marked shift of research to sites in Spain, Italy, Belgium, Japan, Israel, Austria, Taiwan and Argentina (714, 700, 301, 314, 212, 185, 153 and 122 additional sites opened, respectively), suggesting these are preferred countries when sponsors face an increased enrolment need.

**Conclusion:** East Asia, Japan and the Middle East are increasingly involved in diabetes trials, while other “emerging” regions seem to lose terrain. In more than 40% of initial trial records final enrolment number or trial duration are underestimated. Some countries appear to be preferred when additional sites are required for a running trial.

## 1015

### Health care climate questionnaire: a useful tool to assess the health care provider approach as perceived by parents of children with diabetes

C.E. de Beaufort<sup>1,2</sup>, K. Lange<sup>3</sup>, S.E. Skovlund<sup>4</sup>, H. Hoey<sup>5</sup>, L. Castano<sup>6</sup>, H. Mortensen<sup>7</sup>, Hvidoere Study Group;

<sup>1</sup>DECCP, Clinique pédiatrique, Luxembourg, <sup>2</sup>Lcsb, University of Luxembourg, Esch sur Alzette, Luxembourg, <sup>3</sup>Department of Medical Psychology, Medizinische Hochschule, Hannover, Germany, <sup>4</sup>Novo Nordisk A/S, Gentofte, Denmark, <sup>5</sup>Trinity College, Dublin, Ireland, <sup>6</sup>University of Basque Country, Barakaldo, Spain, <sup>7</sup>Department of Pediatrics, Herlev Hospital, Denmark.

**Objective:** Previous studies have suggested that health care partnership supporting autonomy may improve outcome in patients with chronic illness. In the Hvidoere Study 2009, we analysed whether the health care climate perceived by parents, influenced wellbeing in parents and outcome in children.

**Methodology:** In this cross-sectional study, children with type 1 diabetes mellitus treated in 18 centres, (Australia, Europe, Israel, Japan and North America) aged <11 yrs and a diabetes duration ≥1 y, participated. Clinical characteristics of the children have been previously reported (n= 1133; female: 47.7 %; mean age 8.0 ± 2.1 y; mean diabetes duration 3.8 ± 2.1 y, CSII/ BBI 49.7%; CT 51.3%, overall mean HbA1c 8.0 ± 1.0% (64 mmol/mol)). The parents were invited to answer questionnaires, including the health care climate questionnaire (HCCQ) and the WHO-5. The HCCQ assesses parents' perception of an autonomy supporting versus controlling approach from the health care providers. They answered the five items on a 7-point Likert-type scale ranging from 1 to 7. WHO-5 is a validated tool to evaluate parental well being.

**Results:** Questionnaires were received from 1015 parents. The Cronbach alpha for HCCQ was 0.82. Relatively high scores were reported in all centres (mean: 6.2 ; range 5.0– 6.9) suggesting autonomy supporting health care. Outcome was not influenced by gender, age, diabetes duration nor diabetes treatment of the child. A weak negative association was found between HbA1c and HCCQ score (r= -0.081, p< 0.008). In those (n= 140) with HbA1c >9, 0%, lower HCCQ scores were found (6.0). WHO-5 score showed a significant relationship with HCCQ score: (r=0.145\*\* p < 0.001), with a lower wellbeing reported by parents with lower HCCQ scores.

**Conclusion:** HCCQ is a valid tool for parents of children with diabetes. Most parents in the Hvidoere centres report an empowerment and autonomy oriented partnership with diabetes teams. Wellbeing as evaluated through WHO-5 was positively associated with this autonomy supporting approach. Worse metabolic outcome seemed associated with lower autonomy support perceived by the parents. Whether these parents would benefit from a different support concept to improve metabolic outcome, requires further study.

*Supported by: Novo Nordisk A/S*

## PS 085 Cost and quality of diabetes care

1016

### Use of diabetes nurse specialist driven telephone service during evenings, nights and weekends by patients attached to a diabetes specialist hospital

M. Due-Christensen, K.E. Nielsen, T. Almdal, V. Zoffmann;  
Steno Diabetes Center, Gentofte, Denmark.

**Background and aims:** Our hospital is a diabetes specialist hospital serving as an integrated part of the public hospital system treating approximately 3600 type 1 diabetes patients (T1D) and 2400 type 2 diabetes patients (T2D). A telephone service staffed with diabetes nurse specialists (DNS) is open for all patients during opening hours of the out-patients clinic. As supplement to the services provided in the out-patient clinic a DNS is on call after-hours 7 days a week concerning diabetes related problems considered critical by patients or their health care professionals. The DNS can, if needed, contact a consultant diabetologist. The aim of the present study was to review the service outside opening hours of the outpatient clinic, i.e. during evenings, nights and weekends concerning the number of calls, the sources and the purposes of the calls as well as the services provided.

**Materials and methods:** All calls to the on-call DNS outside opening hours of the outpatient clinic were registered prospectively by the DNS according to a predefined form in the period from January 21st to July 18th 2011.

**Results:** In total, 3197 calls were registered concerning 592 individual patients (56.4 % of these women), corresponding to approximately 10 % of the patients attached to the outpatient clinic. Age  $56.6 \pm 19.7$  years (mean  $\pm$  SD). 2175 (68 %) calls were related to patients with T1D and 836 (26.2 %) related to patients with T2D (of these 95.7 % were concerning patients treated with insulin). 186 (5.8 %) were related to patients with other types of diabetes. HbA1c  $8.8 \pm 1.9$  (mean  $\pm$  SD), which is significantly higher ( $p < 0.001$ ) than the mean HbA1c of all patients seen in our hospital  $8.1 \pm 1.5$  (mean  $\pm$  SD). 1368 (42.8 %) of the calls were initiated directly by the patients, 1637 (51.2 %) by health care professionals in primary care and 142 (4.4 %) by relatives and 50 (1.6 %) by other sources. Hyperglycaemia was the main purpose for contact corresponding to 1655 (51.8%) calls. Of these 1136 (68.6 %) were related to patients with T1D. The most often provided service was advice in relation to insulin dose adjustment i.e. in relation to hyper or hypoglycaemia or physical activity pertaining 2210 (69.1 %) calls. Of 17 potential admissions, 15 were avoided. One call only to the telephone service was made by 295 (49.8 %) of the patients, whereas 146 (24.7 %) called 2-4 times, 129 (21.8 %) called 5 - 29 times and 22 (3.7%) called 30 times or more (range 30 -162). Approximately 90% of the purposes for calling were considered relevant and related to diabetes by the DNS receiving the call. The DNS handled 3114 (97.4 %) of the calls without supervision from the diabetologist.

**Conclusion:** DNS-provided on-call telephone service outside outpatient opening hours is used by approximately 10 % of the population attached to a diabetes specialist hospital. The most frequent purpose of the calls related to advice on dose adjustment concerning hyperglycaemia. Fifteen admissions were avoided. The service is considered useful for the majority of patients. More investigation is needed concerning patients calling more than 30 times in 6 months in order to improve/learn more about the provision of service to these patients; moreover, a high number of calls came from health care professionals in primary care, suggesting a need for further education concerning diabetes in this sector.

1017

### Cost-effectiveness of intermediate care clinics for diabetes (ICCD): findings of a UK cluster randomised trial

A. Szczepura<sup>1</sup>, P. Saravanan<sup>1</sup>, D. Baines<sup>1</sup>, S. Petrou<sup>1</sup>, R. Crossman<sup>1</sup>, A. Hardy<sup>2</sup>, N. Raymond<sup>1</sup>, A. Wilson<sup>2</sup>, J.P. O'Hare<sup>1</sup>, ICCD Study Group;  
<sup>1</sup>Warwick Medical School, University of Warwick, Coventry, <sup>2</sup>Department of Health Sciences, University of Leicester, UK.

**Background and aims:** World-wide, healthcare systems are faced with an epidemic of type 2 diabetes. In the UK, clinical care is primarily provided by general practitioners (GPs) rather than hospital specialists. Intermediate care clinics for diabetes (ICCD) potentially provide a model for supporting GPs in their care of people with poorly controlled type 2 diabetes. This study aimed

to assess the cost-effectiveness of ICCD provision compare to usual hospital care for such patients.

**Materials and methods:** 49 practices in three English Primary Care Trusts (PCTs) in Coventry, Leicester and Warwickshire were randomised to usual care or access to an ICCD service. All suitable patients were invited to take part. Outcomes were measured after 18 months. The primary clinical outcome was 'combined control' - achievement of all three targets from NICE guidelines of HbA1c  $\leq 7.0\%$ , b.p.  $< 140/80$  mm Hg and cholesterol  $< 4$  mmol/l. Health related quality of life (HRQoL) was measured at baseline and 18 months using EQ-5D. ICCD costs were calculated to include staff, accommodation and consumables. Other NHS resource use was recorded via patient questionnaires. Costs and QALYs were discounted at a rate of 3.5%. Cost-effectiveness was assessed by comparing incremental costs and marginal benefits. Sensitivity analysis was undertaken.

**Results:** A total of 1,997 patients with type 2 diabetes were recruited to the trial. ICCD consultation costs were estimated at £104.33; use of case management increased referral rates and decreased unit costs. GP utilisation costs were higher in the ICCD group (mean £26.56 vs. £ 20.07,  $p = 0.012$ ) as were community clinic costs (£1.46 vs. £ 0.49,  $p = 0.025$ ). Total NHS resource use costs did not differ at the 5% level, whether or not ICCD costs were included. Follow-up data, collected for 1,280 (61%) showed a clinically important gain in combined control (14.3% for ICCD patients vs. 9.3%) despite low numbers of referrals to ICCD in two PCTs. After adjusting for confounding factors and clustering this difference was not significant ( $p = 0.059$ ). There was no significant difference in HRQoL between patient groups at baseline. An incremental cost-effectiveness ratio (ICER) of £7,778, following 1,000 replicated bootstraps, indicated that ICCD is marginally more expensive at producing health gain (EQ-5D) for patients. The cost-effectiveness acceptability curve (CEAC) similarly showed a low probability that ICCD is more cost-effective than standard care at the cost per QALY threshold usually employed by NICE (£30,000). A sensitivity analysis, excluding patients with hospital stay  $> 10$  days, demonstrated a 95.9% probability that the control group remains cost-effective at the £30,000 threshold.

**Conclusion:** Our results indicate that an ICCD service provides no direct cost saving relative to standard care. There is also no evidence that this service model is cost-effective relative to standard care (based on EQ-5D). However, use of case management can increase uptake which might improve cost-effectiveness. Development of a service model to achieve better outcomes for patients with poorly controlled diabetes remains a challenge.

Clinical Trial Registration Number: NCT00945204

Supported by: NIHR SDO Programme

1018

### Diabetes care in a shared care project: trends and age differences in the period 1998-2008 (ZODIAC-19)

K.J.J. van Hateren<sup>1</sup>, I. Drion<sup>1</sup>, N. Kleefstra<sup>1</sup>, K.H. Groenier<sup>2</sup>, S.T. Houweling<sup>1</sup>, K. van der Meer<sup>2</sup>, H.J.G. Bilo<sup>1</sup>;  
<sup>1</sup>Diabetes Centre, Zwolle, <sup>2</sup>University Medical Center Groningen, Netherlands.

**Background and aims:** The Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study was initiated in 1998 to investigate the effects of shared care for patients with type 2 diabetes mellitus in the Netherlands, and to reduce the number of diabetes-related complications. Benchmarking the performance of diabetes care was and is an important aspect of this study. We aimed to investigate trends in diabetes care, within the ZODIAC study for a wide variety of quality indicators during a long follow-up period (1998-2008), with a special focus on different age groups.

**Materials and methods:** A dataset of quality measures is collected annually during the patient's visit to the practice nurse or general practitioner. Linear time trends from 1998-2008 were estimated using linear mixed models in which we adjusted for age and gender. Age was included in the model as a categorical variable: for each follow-up year all participants were categorised into the categories  $< 60$ , 60-75 and  $> 75$  years. Differences in trends between the age categories were investigated by adding an interaction term to the model.

**Results:** Table 1 presents the results of various quality measures for the total study group. All data are mean values or proportions together with their 95% confidence intervals. The number of patients who were reported to participate increased in the period 1998-2008 from 1622 to 27438. All quality indicators improved in this study, except for body mass index. The prevalence albuminuria decreased in an eleven-year-period from 42% to 21%. No relevant differences between the trends for the 3 age categories were observed.



During all years of follow-up, mean blood pressure and body mass index were the lowest and highest, respectively, in the group of patients <60 years (data not shown).

**Conclusion:** Quality of diabetes care within the Dutch ZODIAC study, a shared care project, has considerably improved in the period 1998–2008. There were no relevant differences between trends across various age categories. This study shows the great potential of intensive cooperation between primary and secondary health care.

		1998, n=1622	2000, n=1462	2002, n=1761	2004, n=4729	2006, n=18469	2008, n=27438
Age (years)	mean	68.9 (68.4;69.5)	67.8 (67.2;68.4)	67.0 (66.5;67.6)	67.5 (67.2;67.9)	67.4 (67.2;67.6)	67.4 (67.2;67.5)
	% female	58.0 (55.6;60.4)	56.2 (53.7;58.8)	55.4 (53.1;57.7)	53.8 (52.4;55.2)	52.6 (51.9;53.3)	51.9 (51.3;52.5)
HbA1c (%)	mean	7.5 (7.4;7.5)	7.3 (7.2;7.3)	7.1 (7.0;7.1)	7.0 (7.0;7.0)	6.7 (6.7;6.8)	6.7 (6.7;6.7)
	%<7	40.4 (37.9;43.0)	46.6 (44.0;49.2)	53.3 (50.9;55.8)	57.0 (55.5;58.5)	67.5 (66.8;68.2)	70.1 (69.6;70.7)
SBD (mmHg)	mean	154.5 (153.3;155.8)	149.4 (148.2;150.6)	144.4 (143.4;145.4)	145.9 (145.3;146.5)	141.9 (141.7;142.2)	140.0 (139.8;140.2)
	%<140	22.0 (19.9;24.2)	29.4 (27.0;31.8)	34.6 (32.4;36.9)	37.9 (36.4;39.3)	43.0 (42.2;43.7)	47.7 (47.1;48.3)
Chol-HDL ratio	%<4	23.0 (20.7;25.4)	35.6 (33.1;38.1)	49.8 (47.4;52.3)	59.2 (57.7;60.8)	67.1 (66.3;67.8)	61.1 (60.5;61.7)
	% micro	33.6 (30.8;36.4)	31.4 (28.9;33.8)	25.1 (22.9;27.3)	24.4 (22.9;26.0)	19.2 (18.5;20.0)	18.5 (18.0;19.0)
Albuminuria	% macro	8.3 (6.7;10.0)	6.7 (5.3;8.0)	4.8 (3.7;5.9)	3.9 (3.2;4.6)	2.9 (2.6;3.2)	2.4 (2.2;2.6)
	%<25	20.4 (18.0;22.7)	17.4 (15.5;19.4)	15.8 (14.0;17.5)	16.1 (14.9;17.2)	16.8 (16.2;17.4)	17.1 (16.6;17.6)
BMI	%<25	20.4 (18.0;22.7)	17.4 (15.5;19.4)	15.8 (14.0;17.5)	16.1 (14.9;17.2)	16.8 (16.2;17.4)	17.1 (16.6;17.6)

## 1019

### The value of performance measurement in diabetes

S. Smith<sup>1</sup>, N. Shah<sup>2</sup>, K. Eggleston<sup>3</sup>, V. Montori<sup>1</sup>, J. Newhouse<sup>4</sup>, E. Berndt<sup>5</sup>; <sup>1</sup>Department of Internal Medicine, Mayo Clinic Rochester, <sup>2</sup>Health Care Policy & Research, Mayo Clinic Rochester, <sup>3</sup>Asia Health Policy Program, Stanford University, <sup>4</sup>Department of Health Care Policy, Harvard Medical School, Boston, <sup>5</sup>Applied Economics, MIT Sloan Management, Cambridge, USA.

**Background and aims:** Purchasers are using performance measures to design and implement incentives to improve quality of diabetes care. Haemoglobin A1c (HbA<sub>1c</sub>) has gained wide acceptance as a performance measure, usually based on a threshold value. The aim of this study was to test the assumption that better quality is consistent with better net value.

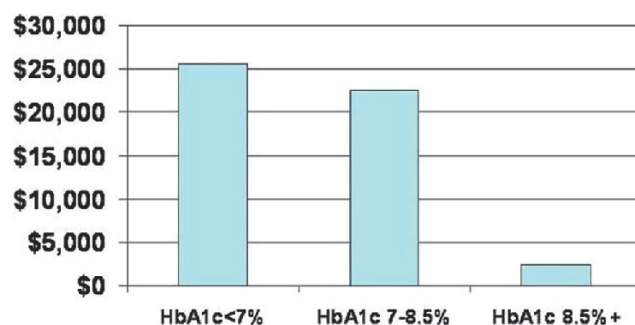
**Materials and methods:** Using data from Mayo Clinic Rochester's Electronic Management System, we identified 970 patients diagnosed with type 2 diabetes before 1 January 2005 who were continuous Mayo employees or dependents of employees from 1999 to their deaths or to 2009. We assess the net value of diabetes management, defined by whether the value of prevention of future mortality and morbidity exceeds the increase in costs of diabetes management. Using the United Kingdom Prospective Diabetes Study cardiovascular risk equations, we estimate the 10-year modifiable risk of coronary heart disease (CHD) or stroke for each patient. "Modifiable" risk holds constant the patient's age and duration of diabetes. We compared changes in these 10-year risks for nine "HbA<sub>1c</sub> cohorts" based on initial and final HbA<sub>1c</sub> values: <7%, 7 to 8.5%, and greater than 8.5%. Spending is for all medical care, related- unrelated to the diagnosis of diabetes, converted to constant 2009 dollars using GDP deflator.

**Results:** Estimated CHD risk improved, with all HbA<sub>1c</sub> cohorts experiencing a decrease except for patients with final HbA<sub>1c</sub> greater than 8.5%. All cohorts experienced small decreases in the estimated risk of fatal stroke. Mean annual inflation-adjusted spending for the care of patients with type 2 diabetes increased for all HbA<sub>1c</sub> cohorts, although those patients with HbA<sub>1c</sub> values under 7% in both time periods had one of the most modest increases (18%). Assuming a monetary value of \$200,000 for one life-year, net value was positive for all HbA<sub>1c</sub> cohorts except patients with poor control throughout (HbA<sub>1c</sub> greater than 8.5% in both time periods). The highest net value, of over

\$52,815, was for the cohort with the largest improvement in HbA<sub>1c</sub> (>8.5% to <7%). These results suggest good value for keeping patients below 8.5%. (Figure)

**Conclusion:** On average, the monetary value of the patients' improvements in cardiovascular risk exceeded health spending for cohorts with final HbA<sub>1c</sub> values less than 8.5%. While it may appear to be consistent with a threshold that could be used for determining pay-for-performance, it is a higher value than most stated guidelines and current measures for pay-for-performance. It suggests that other factors in the population are likely contributing to outcomes and are worthy of attention for improving value (e.g. access to care, smoking cessation, depression, comorbidities).

### Figure Net Value, by HbA1c in Final Period



## 1020

### The quality of care provided by Italian diabetes clinics to elderly patients with type 2 diabetes

R. Candido<sup>1</sup>, M.A. Pellegrini<sup>2</sup>, G. Felace<sup>3</sup>, M. Boemi<sup>4</sup>, A. Perrelli<sup>5</sup>, M.C. Rossi<sup>6</sup>, A. Nicolucci<sup>6</sup>, C. Giorda<sup>7</sup>; <sup>1</sup>Diabetes Centre, A.S.S. 1 Triestina, Trieste, <sup>2</sup>Diabetes and Metabolism Unit, A.O.U. Santa Maria della Misericordia, Udine, <sup>3</sup>Diabetes and Metabolism Unit, O.C. di Spilimbergo, Udine, <sup>4</sup>Diabetes Unit, Department of Medicine, INRCA – IRCCS, Ancona, <sup>5</sup>Diabetes Unit, ASL NA 2 NORD, Napoli, <sup>6</sup>Department of Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud, S. Maria Imbaro, <sup>7</sup>Diabetes and Metabolism Unit, ASL TO5, Chieri, Italy.

**Background and aims:** The prevalence of diabetes in people over 65 years approaches 20%, while at least 50% of all patients with diabetes are older than 65 years. Clinicians who manage older people with diabetes require special skills to provide high-quality care. In the context of a continuous quality improvement initiative promoted in Italy by Associazione Medici Diabetologi (AMD Annals initiative), we evaluated the quality of care provided by diabetes clinics to young old (i.e. 65–74 years) and old patients (i.e. >75 years) as compared to younger ones.

**Materials and methods:** Overall, 251 diabetes clinics (one third of all clinics in Italy), all using electronic medical record systems, extracted data relative to the year 2009. The proportion of patients with at least one value registered during the year (process measures), the percentage of patients reaching specific favourable or unfavourable targets (intermediate outcome measures), and rates of use of drugs were evaluated. In addition, a quality of care summary score (Q score) was calculated. The Q score, ranging between 0 and 40, is based on process and outcome indicators (HbA<sub>1c</sub>, blood pressure, LDL-cholesterol, microalbuminuria) and is closely related to long-term outcomes in diabetic patients.

**Results:** Overall, 414814 patients were evaluated, of whom 34.9% were aged 65–74 years and 25.2% ≥75 years (mean age 70.0±2.8 and 80.3±3.9, respectively vs. 55.8±7.8). As compared to younger patients, young old and old individuals were less often males (54.8 and 45.2 % vs. 57.9%), had lower BMI (29.5±5.0 and 28.3±4.6 vs. 30.0±5.4 Kg/m<sup>2</sup>), longer diabetes duration (11.3±9.3 and 14.1±10.9 vs. 9.3±8.5 years), and were less likely to smoke (13.8% and 7.2% vs. 20.3%). Table 1 shows quality of care indicators.

	Indicator	<65	65-74	≥75
Process	HbA <sub>1c</sub>	92.5	93.3	91.2
	Blood pressure	80.0	80.3	74.8
	Lipid profile	75.1	75.2	68.1
Favourable outcome	Microalbuminuria	42.8	42.9	37.0
	HbA <sub>1c</sub> ≤7%	43.4	45.0	42.5
	BP ≤130/80 mmHg	17.5	13.5	13.7
	LDL-C <100 mg/dl	38.2	44.4	43.9
Unfavourable outcome	HbA <sub>1c</sub> ≥9%	16.5	10.8	10.9
	BP ≥ 140/90 mmHg	51.1	60.3	62.1
	LDL-C ≥130 mg/dl	29.6	23.5	23.5
Treatment	Oral agents:			
	Metformin	62.0	56.0	42.1
	Sulfonylureas	31.8	36.6	36.2
	Insulin	11.4	15.6	23.4
	≥2 antihypertensive agents	14.8	23.6	25.1
	Lipid-lowering agents	39.7	45.8	37.4
Global	Q SCORE <15	8.3	7.3	7.7
	Q SCORE >25	37.2	37.6	33.1

**Conclusion:** Care provided by diabetes clinics to elderly patients seems to be different compared to younger individuals. Elderly patients are less frequently monitored for blood pressure, lipid profile and microalbuminuria, but show slightly better intermediate outcomes and are more often treated with sulfonylureas and insulin. Despite a lower Q score, intensity of treatment does not seem to be affected by patient age.

## 1021

### An innovative community health worker training program: addressing the diabetes pandemic through expansion of the diabetes health care team

K.M. Collieran<sup>1</sup>, E. Harding<sup>1</sup>, A. Zurawski<sup>1</sup>, D. Somm<sup>1</sup>, B. Kipp<sup>2</sup>

<sup>1</sup>Internal Medicine, University of New Mexico HSC, Albuquerque,

<sup>2</sup>President, Blackfeet Community College, Blackfeet Tribe, USA.

**Background and aims:** Diabetes is rapidly becoming a pandemic. Health care systems are ill-equipped to meet the demands of the impending global diabetes crisis. Rapid expansion of the diabetes work force and mobilization of diabetes care teams will be essential to prevent devastating morbidity, mortality and financial disaster. Lay health workers serve as health care extenders and patient advocates across the world. They are effective in their mission due to their dedication, low cost, cultural, linguistic, and community understanding of the patients they advocate for. We hypothesize that community health workers (CHWs) can be trained using a distance learning model to become diabetes health extenders to increase the diabetes work force and expand the diabetes care team in a culturally appropriate, low cost, and effective manner. **Materials and methods:** We devised a multi-modality diabetes training program for CHWs that included a three day hands on intensive didactic, clinical skills and communications training, along with asynchronous video modules. Subjects then participated in a 6-month weekly distance case based and didactic learning program, using internet and video conferencing. Subjects completed evaluations at baseline and following the 6-month program. Surveys used included the Michigan Diabetes Attitude Survey (DAS-3), a modified version of the Michigan Diabetes Knowledge Test (mDKT), and a Diabetes Confidence Survey (DCS) for both clinical and nonclinical skills for diabetes. Subjects also completed 1:1 pre and post participation evaluations to assess mastery of specific diabetes related clinical and knowledge skills. Finally supervisors were asked to evaluate the effect the training had on their organisations and diabetes programs. Data were analysed using paired students t tests. Significance (p) and effect size were determined (d).

**Results:** Fifty CHWs completed the program including 4 men and 46 women; 30 Native Americans, 12 Hispanics, and 7 Whites. Subjects improved in all five domains of the DAS-3, but statistical significant improvements were only seen in the Seriousness and Patient Autonomy scales (p<0.01 and <0.05, d=0.41 and 0.38 respectively). Participation in the program resulted in a significant improvement in the mDKT mean percent correct (pre 58.2% to post 68.4 % (p < 0.0001, d = 0.76). Significant improvements in confidence in clinical and non clinical skills were seen from pre to post testing (p <0.0001, d>0.9).

All domains of clinical skills testing improved significantly including blood pressure, blood sugar, and BMI measurement and interpretation, depression screening, diabetic foot exam, and medication counseling. Finally, supervisors reported significant improvements in CHW's skills (composite score of 18 parameters) and integration into the diabetes care team as a result of participation in the program (p<0.001, d=1.7, and p<0.001 d=1.78, respectively).

**Conclusion:** We have demonstrated that our innovative training program led to significant improvement in CHW attitudes, knowledge, clinical skills and confidence in their abilities in these areas. Additionally, their supervisors recognized the value of the training and increased CHW responsibility and integration in diabetes care teams. Specialized training of CHWs in diabetes is a potential mechanism to help address the global diabetes epidemic.

Supported by: RWJ

## 1022

### Quality management in care groups and outpatient clinics in the Netherlands

M.J.E. Campmans-Kuijpers<sup>1</sup>, L.C. Lemmens<sup>2</sup>, C.A. Baan<sup>3</sup>, K.J. Gorter<sup>1</sup>,

J. Groothuis<sup>3</sup>, K.H. van Vuure<sup>3</sup>, G.E.H. Rutten<sup>1</sup>;

<sup>1</sup>Julius Center, University Medical Center Utrecht, <sup>2</sup>National Institute for Public Health and the Environment, Bilthoven, <sup>3</sup>Knowledge Centre for Chain Care, Zwolle, Netherlands.

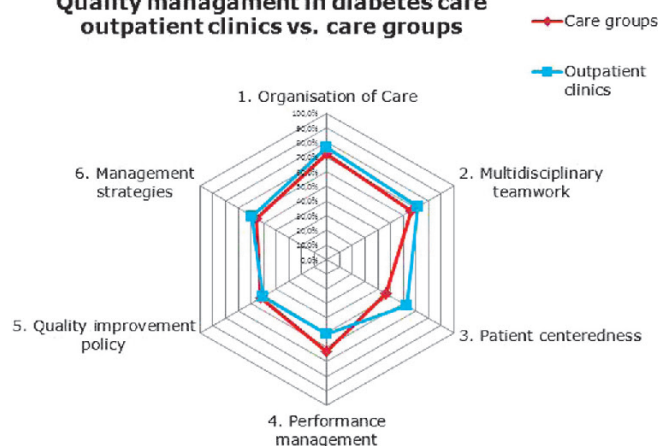
**Background and aims:** In the Netherlands, standard diabetes care is provided either in diabetes care groups (n=97) or outpatient clinics (n=104) using a diabetes care program. The organisation or care groups varies and is still under development. Quality of care indicators have been addressed enormously during the last years. However, it is essential to understand the comprehensive elements needed for good quality management and assurance of care at organisational level too. The aim of this study is to investigate in what way the above mentioned organisations organise quality management. In January-March 2012 we measured the actual level of quality management of care for T2DM patients in care groups and outpatient clinics nationwide.

**Materials and methods:** Based on current literature online questionnaires for quality managers were developed for measuring the level of quality management in outpatient clinics and in care groups separately. Results of questionnaires were plotted in a radar diagram consisting of six domains of quality management. Scores in the domains (0-100%) and sub domains were compared with the mean score in care groups or outpatient clinics.

**Results:** Totally 203 quality managers were invited to fill out the online questionnaire (97 care groups, 104 outpatient clinics). Responders of 60 care groups (response rate 61.9 %) showed a mean score of 59.4% (median 60.3, range 39-77%). Responders on behalf of 52 outpatient clinics (50%) had a mean score of 61.9% (median 59.7%, range 27-89%). The six domains gave the following mean scores for care groups and outpatient clinics respectively: organisation of care 71.9%, 76.8%; multidisciplinary teamwork 66.6%, 71.5%; patient centeredness 46.3%, 62.5%; performance management 64.0%, 50.9%; quality improvement policy 52.7%, 50.9% and management strategies 56.0%, 59.0%. Results in sub domains revealed that outpatient clinics have a low score on the patients' right to scrutinize of their medical file (28.0%) and patient involvement (26.8%), and care groups hardly focus on patient involvement (17.2%) and patient safety (15.3%).

**Conclusion:** This nationwide overview of diabetes quality management policy shows reveals that groups focus more on performance management and less on patient centeredness, whereas outpatient clinics focus less on performance management and more on patient centeredness.

### Quality management in diabetes care outpatient clinics vs. care groups



Supported by: Dutch Diabetes Federation (grant no NAD 3.05)

## 1023

### The novel use of commonly captured data to assess a district's diabetes service that evaluates both primary and secondary care

E.C. Russell-Jones<sup>1</sup>, A. Gough<sup>2</sup>, I.N. Scobie<sup>2</sup>;

<sup>1</sup>Medicine, GKT Medical School, King's College London, <sup>2</sup>Diabetes and Endocrinology, Medway Maritime Hospital, Medway, UK.

**Aims:** There is a need to assess performance of diabetes care that encompasses both primary and secondary care in the UK. None of the quoted schemes do this. However data are routinely collected and freely available that would allow a new index to be created that looks at both primary and secondary care within a district or region. The routinely collected data include: 1. Primary care QOF (Quality outcomes framework) information/data for diabetes (a marker of primary care performance). 2. Referral rates for first appointment for specialist secondary care review, a marker of how often secondary care help is being sought (Dr Foster/ HES Hospital Episode Statistics). 3. Emergency admission rates for diabetes (markers of how often emergency treatment is needed from primary care). (Dr Foster/HES).

**Methods:** To investigate this new index, a district diabetes service (Medway PCT (300,000 population) that encompasses both rural and urban communities) was studied. The most illustrative measures from QOF were total points (sum of all diabetes indicators) and DM23 attainment of HbA1c <7% combined with its exemption reporting rate (number of patients excluded by each practice from analysis). Performance was compared to the other 8 PCTs within the South East Coast SHA. Dr Foster data were used that included SAR (Standardised admission rate) for first or new OPD referral to specialist diabetes services and SAR for emergency diabetes admissions to hospital.

**Results:** NHS Medway has the highest prevalence of Diabetes at 6.1% of all the 8 PCTs in the SHA. Medway has a lower achievement of diabetes QOF points (96.1%) relative to other PCTs. Medway has the lowest achievement of an HbA1c level <7% (54.3%) across all the PCTs within the SHA. Exemption reporting was also 3<sup>rd</sup> highest within the SHA. SAR for first/new diabetes OPD appointment to the hospital was very low at 281, (predicted 576) 48% of expected. The emergency admission rate was high at 225 (predicted 168), producing an emergency admission SAR of 133% expected.

Title	Medway	SHA Average	Number	Expected	SAR Standardised admission rate
Prevalence of Diabetes %	6.1	5.1			
Total QOF DM points	96.2	96.6			
DM23 % attaining HbA1c <7%	54.3	55.8			
Exemption % DM 23	14.2	13.7			
First/new OPD referral to Hospital			281	576	48%
Emergency Admission to Hospital			225	168	133%

This new index shows that Medway district has a high prevalence of diabetes, a relatively low attainment of primary care QOF diabetes points and high exception reporting rate. The referral rate for new diabetes referrals to specialist OPD is low in comparison to the national average, and there is a considerably higher emergency admission rate. Thus primary care diabetes needs more resources and support to raise performance and there should be a lower threshold for OPD referral of complex patients to specialist hospital clinics to prevent/lower emergency admissions.

**Conclusion:** It is possible to combine routinely available data to produce an assessment of diabetes care that transcends primary and secondary care (applicable anywhere in UK) that gives a true reflection of a district's performance which will be useful to plan future health provision.

## 1024

### Risk factors for dropping out of a disease management programme (DMP). Results from the DMP diabetes mellitus type 2 in the North Rhine region, Germany

L. Altenhofen, B. Hagen, J. Kretschmann, A. Weber;  
Central Research Institute for Ambulatory Health Care in Germany, Cologne, Germany.

**Background and aims:** Published evidence with regard to a possible selection bias when entering a DMP for the indication of diabetes mellitus type 2 is inconsistent. So far no systematic analyses exist which examine the differences between patients who stay in or who drop out of the programme. For the North Rhine region, Germany, the group of drop out patients is described quantitatively. A statistical model of the DMP drop out risk is developed.

**Materials and methods:** Until the end of 2010 423 534 patients took part in the DMP. In contrast until the end of 2009 33 321 former patients did drop out of the programme. According to administrative data (limited availability) 5 699 of these 33 321 patients died. So a total of 27 622 drop out patients was left for analyses. As a first step data were analysed by descriptive statistics. As a second step the risk for dropping out of the DMP was calculated via logistic regression analysis (odds ratios and 95 percent confidence intervals are given).

**Results:** With regard to the last documented data of both populations in 2009 by comparison to patients who continued participation patients who left the DMP were on average one year older ( $67.8 \pm 14.2$  vs.  $66.5 \pm 11.6$  years) and did suffer longer from diabetes ( $10.0 \pm 7.8$  vs.  $8.4 \pm 7.0$  yrs.). Average hba1c percentage was slightly increased among patients who left the DMP ( $7.2 \pm 1.5$  vs.  $7.0 \pm 1.2$ ). In addition frequencies of missed patients' education were higher among patients who left the DMP (15.4 vs. 12.3 %). In the multivariate model older age (76 yrs. or more vs. 65 yrs. or less, OR 1.71, CI 1.62-1.81), incidence of difficult comorbidities (blindness, dialysis, amputation, OR 1.73, CI 1.49-2.01), hba1c percentage of 8.5 or more (OR 1.83, CI 1.72-1.94) as well as inpatient diabetic care (OR 1.58, CI 1.26-1.99) were the most significant risks for dropping out of the DMP. Large correlations were observed too with missed patients' education (OR 1.35, CI 1.29-1.41) and missed eye examination (OR 1.96, CI 1.86-2.05).

**Conclusion:** Patients who drop out of the DMP are older, suffer longer from diabetes, require more often inpatient care and suffer more often from difficult comorbidities. In addition the larger risks of missed patients' education and eye examination can be a sign of a lower level of therapeutic adherence among these patients. Actually patients who prematurely leave the DMP are an important target group of a structured care programme. Therefore thoroughly organised additional studies of the DMP are urgently needed to ex-



amine the different individual reasons for dropping out of the programme. These reasons should be verified too in other German regions where DMPs are implemented.

## 1025

### Cost of diabetes care and its association with good glycaemic control: a hospital based study

A. Afroz<sup>1</sup>, M. Shahjahan<sup>1</sup>, M. Hafez<sup>1</sup>, L. Ali<sup>2</sup>;

<sup>1</sup>Department of Biostatistics, Bangladesh Institute of Health Sciences,

<sup>2</sup>Department of Biochemistry & Cell Biology, Bangladesh Institute of Health Sciences, Dhaka, Bangladesh.

**Background and aims:** Diabetes is creating a major socio-economic burden on individual, societal and state levels, and cost-effectiveness of interventions against this disorder is a high priority particularly in developing countries. Primary evidence on these issues are scarce even in developed world and these are almost absent in the developing countries. The present study was undertaken to assess the cost-effectiveness of good glycemic control in a Bangladeshi type 2 dietetic population.

**Materials and methods:** A cross-sectional study was conducted among 496 registered patients (aged  $\geq 30$  years) of Out-Patient Department of the central hospital of Diabetic Association with more than 1 year duration of diabetes. Effectiveness of treatment was judged by good glycemic control with FSG < 6.1 mmol/L as the cut-off value. All treatment related records of the last 1 year were collected from patients' guide books. The degree and extent of complications like cardiopathy, retinopathy, nephropathy and vasculopathy were recorded and direct, indirect & incremental cost of the management of diabetes and complications were calculated from consumers' point of view. Direct cost included consultation fees, drugs, laboratory investigation and other medical services. Indirect cost included opportunity cost of participants and attendance calculated by human capital approach.

**Results:** Among 496 patients 46% were males and 54% were females with mean  $\pm$  SD duration of diabetes  $8.8 \pm 6.8$  years. Among them 31% have good glycemic control. The average annual cost of care was US\$ 313 (direct US\$ 283 & indirect US\$ 31) per patient. Drugs accounted for the largest share of direct cost US\$ 193 (67%), followed by laboratory investigations US\$ 27 (13%) and consultation fees US\$ 23 (12%). Average annual cost of participants with good glycemic control was US\$ 303 and with poor glycemic control it was US\$ 318; the difference was statistically significant ( $p < .05$ ). Participants with the poor glycemic control were significantly more likely to have complications [( $p = .049$ ) OR 1.5 (95% CI 1.0–2.3)] and co-morbidities [( $p = .02$ ) OR 1.5 (95% CI 1.0–2.2)] than participants with good glycemic control. Participants with both cardiopathy-retinopathy had 2.3 times, both cardiopathy-nephropathy had 1.8 times and both nephropathy-retinopathy have 3.4 times higher cost of care compared to participants without complications. The annual medical costs of good glycemic control participants increased with increase in the number of complications/co-morbidities. Without complications/co-morbidities spent US\$ 271 but the cost increased to US\$ 294 with one/two and US\$ 378 with more than two. The annual cost of poor glycemic participants increased rapidly with complications/co-morbidities: US\$ 276, US\$ 318 and US\$ 419 for without complications/co-morbidities, one/two and more than two respectively. Multiple regression analysis showed that number of complications/co-morbidities ( $p = 0.01$ ) and duration of diabetes were significant ( $p = 0.01$ ) explanatory variables of annual cost of care ( $R^2 = 0.16$ ;  $F = 23.9$ ,  $P < 0.001$ ).

**Conclusion:** The average annual cost of diabetes care per patient in Bangladesh is US\$ 313 and with an estimated 9.5 million diabetic patients, the total annual burden will be US \$ 2973.5 million. Good glycemic control can lead to substantial cost saving through prevention and control of complications.

Supported by: BADAS

## PS 086 Pumps and new devices

### 1026

#### Efficacy and safety of insulin pump treatment in young adult patients with type 1 diabetes mellitus

T. Klupa, B. Matejko, K. Cyganek, M. Grzanka, E. Kozek, M.T. Malecki; Department of metabolic Diseases, Jagiellonian University, Krakow, Poland.

**Background and aims:** Continuous subcutaneous insulin infusion (CSII) via personal insulin pumps is a valuable therapeutic tool in T1DM patients. However, it is not clear whether this treatment is equally effective in different patient subgroups. Adherence to recommended CSII-related behaviors may especially be of concern in young adults with intensive, variable daily activities (students, young professionals). The aim of this observational study was to estimate treatment outcomes in young adult patients with T1DM and compare them with older individuals.

**Materials and methods:** Overall 140 adults with T1DM on CSII were examined; they were divided into two subgroups: 77 patients younger than 26 years of age (range 18–26 years, mean 20.6 years) and 63 older subjects (range 26.5–75.5 years, mean 39.0). The younger subgroup was characterized by shorter diabetes duration (10.0 years vs. 17.7 years,  $p < 0.00001$ ). The groups did not differ with respect to BMI (22.8 kg/m<sup>2</sup> in young T1DM subjects vs. 23.7 kg/m<sup>2</sup> in older patients,  $p = 0.07$ ). Individuals with shorter than a 3-month history of CSII treatment were excluded from the analysis. We compared glycemic control in both groups of T1DM subjects and analyzed treatment attitudes to identify potentially modifiable behaviors influencing the treatment efficacy. The available insulin pump and blood glucose meter downloads as well as HbA1c levels were reviewed. We analyzed records of insulin pumps from the last 4–6 weeks, while for glucometers the whole memory content (200–300 records depending on glucometer type) was included.

**Results:** Younger individuals were characterized by significantly worse treatment outcomes as compared to the older population: mean HbA1c levels were  $7.6 \pm 1.3\%$  and  $6.9 \pm 1.3\%$  ( $p = 0.00001$ ), while mean glucose levels based on glucometer downloads were  $161 \pm 33.6$  mg/dL and  $136 \pm 21.8$  mg/dL ( $p = 0.00001$ ), respectively. Of interest, there were no differences in glucose variability (SD/x mean  $\times 100\%$ ) between the two groups ( $46.7 \pm 9.1\%$  vs.  $44.3 \pm 8.2\%$ , for younger and older individuals, respectively,  $p = 0.1498$ ). The number of hypoglycemic episodes (defined as glycemia  $< 55$  mg/dL) for 100 measurements was higher in older vs. younger patients ( $6.2 \pm 4.9$  vs.  $4.5 \pm 4.0$ ,  $p = 0.0352$ ).

**Conclusion:** The efficacy of CSII treatment observed in young T1DM adults was worse as compared to older patients. Possible reasons include lower SMBG frequency and less frequent use of advanced insulin pump options. The higher number of hypoglycemia/100 measurements in older individuals may be due to stricter glycemic control or to a higher number of SMBG measurements in this group.

### 1027

#### Identifying characteristics of insulin pump use that predict good diabetes control in patients with type 1 diabetes

S. Sivasubramaniyam<sup>1</sup>, L. Green<sup>1</sup>, S.A. Amiel<sup>1</sup>, P. Choudhary<sup>1</sup>;

<sup>1</sup>Diabetes Research Team, King's College Hospital, London, UK.

**Background and aims:** Continuous subcutaneous insulin infusion (CSII) therapy is an increasingly common treatment option for type 1 diabetes. However, a proportion of patients fail to achieve glycaemic targets despite CSII. We examined differences in pump set-up and usage characteristics between patients with target and sub-optimal glycaemic control as well as patients with low and high rates of hypoglycaemia.

**Methods:** All adult patients with type 1 diabetes treated with CSII at a single hospital clinic were considered ( $n = 454$ ). Exclusion criteria were CSII treatment  $< 6$  months, pregnancy, haemoglobinopathy, continuous sensor use and lack of pump download in past year. 198 patients meeting these criteria were categorised into those with target [HbA1c  $< 7.5\%$  ( $n = 52$ )] and sub-optimal [HbA1c  $\geq 7.5\%$  ( $n = 146$ )] glycaemic control. Patients were also divided into those with high [ $\geq 3$  episodes/week ( $n = 76$ )] and low [ $< 3$  episodes/week ( $n = 105$ )] rates of hypoglycaemia [defined as capillary glucose  $< 4$  mmol/L]. Capillary glucose readings, pump set up and usage characteristics were obtained from the last available pump download with concurrent HbA1c obtained within a month.

**Results:** There were no significant differences in baseline characteristics [age, gender, ethnicity, duration of CSII, days of download data] between the target

and sub-optimal glycaemic control group except in mean weight [72.3(±11.6) vs 78.1(±14.3) kg  $p=0.018$ ] and hence BMI [25.5(±3.6) vs 27.2(±4.5) kg/m<sup>2</sup>  $p=0.03$ ]. Similarly, there were no significant differences in baseline characteristics between those with low and high rates of hypoglycaemia. Patients with target glycaemic control used more basal rates [5.57(±2.6) vs. 4.84(±1.8);  $p=0.029$ ] and boluses per day [6.1(±2.1) vs. 5.2(±2.1);  $p=0.004$ ] and had lower insulin to carbohydrate ratios [1.17(±2.8) vs. 1.36(±2.5) u/10g CHO;  $p=0.02$ ]; but there were no differences in total insulin/day, total daily dose/kg or proportion basal insulin than those with sub-optimal control. Target control group had more frequent hypoglycaemia [4.1(±3.6) vs. 2.7(±2.8) episodes/week;  $p=0.009$ ]. Patients with high rates of hypoglycaemia tested blood glucose more often [6.4(±2.1) vs. 3.94(±2.0)/day;  $p<0.0001$ ], gave more boluses [5.75(±1.8) vs. 5.15(±2.1);  $p=0.018$ ] and used the bolus calculator more [4.89(±2.1) vs. 4.1(±2.4);  $p=0.009$ ] but also overrode the bolus calculator more frequently [16.7(±19.5) vs. 13.7(±20.1)%;  $p=0.02$ ]. Post-hoc analysis showed that these differences were irrespective of glycemic control grouping. Patients in the low hypo rate group had significantly higher average blood glucose [10.7(±3.0) vs. 8.8(±1.4) mmol/L;  $p<0.0001$ ] and HbA1c [8.3(±1.1) vs. 7.8(±1.1)%  $p=0.003$ ].

**Conclusion:** Target glucose control on CSII was associated with higher number of basal rates and a greater number of boluses per day suggesting greater engagement with the pump, and more pro-active management of glucose control. However, target HbA1c was also associated with greater biochemical hypoglycaemia. Irrespective of glucose control, increased hypoglycaemia was associated with more frequent capillary testing, suggesting increased vigilance. Further studies exploring pump usage characteristics that results in optimal glycaemic control without problematic hypoglycaemia are merited.

## 1028

### Trends in the treatment of type 1 diabetes with CSII vs MDI: a Canadian retrospective observational study

J. Alfonso Ross Terres, A.D. Jabbour, M.A. Freeman;  
Medical, GSK, Mississauga, Canada.

**Background and aims:** Patients with type 1 diabetes mellitus (T1DM) are treated with insulin via continuous subcutaneous insulin infusion (CSII) or multiple daily injections (MDI). CSII is becoming a more common modality of insulin delivery, as improvements in glycemic control, reductions in glucose fluctuations and reduced rates of severe hypoglycaemia are reported in published literature. The purpose of this observational study was to follow a Canadian cohort of T1DM patients over time to better understand the use of CSII and MDI and the rate of switching between the two insulin modalities.

**Materials and methods:** A national pharmacy-based database (IMS Brogan) was utilized to identify a cohort of T1DM patients, followed from 2008 to 2010. Patients with a prescription history of oral antidiabetic drugs, long-acting/premix insulin monotherapy, inconsistent reporting of data in the last 58 months or fewer than 6 prescriptions were excluded from the cohort. Those without data over the full 2-year study period were excluded from the analysis. Patients were categorized by treatment modality based on insulin prescriptions. CSII users were defined as patients on rapid analog insulin only and MDI users were defined as those on a combination of rapid and long acting insulin. Those not categorized as CSII or MDI users were classified as Other. The cohort was followed over a 2-year period to assess switching between treatment modalities.

**Results:** A cohort of 47,396 T1DM patients was identified in 2008; 39,800 (84%) were classified as MDI patients, 5,858 (12%) as CSII and 1,738 (4%) as Other. After 2 years of follow-up, 36,550 (92%) of the MDI patients continued on MDI modality, 2,567 (6%) switched to CSII, 494 (1%) switched to Other and <1% returned to MDI after switching over the 2 years. Of the 5,858 patients in the CSII group, 4,819 (82%) continued on CSII, 844 (14%) switched to MDI, 58 (1%) switched to Other and 137 (2%) returned to CSII after switching over the 2 years. Of the 1,738 patients in the Other group, 1,056 (61%) continued on Other, 572 (33%) switched to MDI, 91 (5%) switched to CSII and 19 (1%) returned to Other after switching over the 2 years. Of the patients switching treatment modalities in 2009, the overall most common change was MDI to CSII, representing 47% of switches; this increases to 55% of switches in 2010. Although the majority of patients included in this study were ≥ 40 years old (64%), switching from MDI to CSII was more common in patients aged <20 years (16%) vs. ≥20 years (5%).

**Conclusion:** Over 2 years of follow-up, the majority of the T1DM cohort used MDI treatment modality and did not switch. A trend towards the adoption of CSII was observed. Switching from MDI to CSII was most common among younger patients. The limitations of the analysis were the short study

period and inability to adjudicate the CSII and MDI categorization. Further research is necessary to understand the potential long term benefits of CSII in comparison with MDI.

## 1029

### Clinical assessment of DIAAdvisor device shows high accuracy in glucose prediction at 20-min horizon and a coherence of most advices on therapy in patients with type 1 diabetes

A. Farret<sup>1</sup>, E.M. Renard<sup>1</sup>, J. Place<sup>2</sup>, M. Mindlova<sup>3</sup>, E. Vavrova<sup>3</sup>, F. Saudek<sup>3</sup>, M. Vedovato<sup>4</sup>, A. Maran<sup>4</sup>, A. Avogaro<sup>4</sup>, DIAAdvisor consortium;

<sup>1</sup>Department of Endocrinology, Diabetes, Nutrition, Montpellier University Hospital, <sup>2</sup>Department of Endocrinology, Diabetes, Nutrition, University of Montpellier I, France, <sup>3</sup>IKEM, Prague, Czech Republic, <sup>4</sup>University of Padova, Italy.

**Background and aims:** The DIAAdvisor device has been designed in view of predicting blood glucose at a close time horizon and delivering advices on correction measures in order to maintain blood glucose in a near-normal target range in patients with type 1 diabetes (T1D) using a basal-bolus insulin regimen by multiple daily insulin injections or insulin pumps. Inputs include patient information on carbohydrate intakes, insulin dose administration and continuous glucose monitoring (CGM) data. In DIAAdvisor-1 study, we investigated the accuracy of blood glucose prediction at a 20-min horizon and the coherence of treatment advices in various life conditions.

**Materials and methods:** Twenty-nine T1D patients volunteered to assess DIAAdvisor device in three Clinical Research Centres at Montpellier (MPL), Prague (IKEM) and Padova (PAD) for 2 days at rest. Among them, 7 were also submitted to an exercise bout (MPL), 9 to induced hyper- and hypoglycaemic conditions (IKEM) and 8 to a large meal challenge (PAD). The device includes an Ultra Mobile (UM) PC with a patient interface and a USB connection to a CGM DexCom Seven-Plus receiver. Algorithms for glucose prediction and treatment advice are uploaded on the UMPC. Predictions and advices were blinded to the patient but wirelessly transmitted to a nearby clinician monitoring station. The primary endpoints and success criteria were: (i) accuracy of blood glucose prediction at a 20-min horizon assessed by using Clarke error grid analysis of paired predicted glucose values and YSI glucose measurements with > 80% of paired points in A+B zones and <5% in E zone (>75 and <10%, respectively, during challenges) and, (ii) advices given by the device on glucose and insulin corrections with > 80% coherence with clinician's advices.

**Results:** For 48 hours at rest, the accuracy of glucose prediction at 20 min was validated as shown by 89–100% of paired points in A+B zones and 0% in E zone in 28 out of 29 patients. One single case showed 13% of paired points in E zone. Success criteria for prediction were met during the various challenges in all patients except one who showed only 73% of paired points in A+B zones during a large meal test. The coherence of advices at rest reached 96.5, 80.8 and 88.6% with 85.7, 88.0 and 94.5 % full coherence, in MPL (n=11), IKEM (n=9) and PAD (n=9), respectively. During the challenges, the coherence of advices was 87.6, 91.3 and 91.4% with 82.0, 89.9 and 85.4% full coherence, in MPL, IKEM and PAD, respectively.

**Conclusion:** This pilot study shows that the DIAAdvisor device is able to provide successful prediction of blood glucose at a 20-min horizon and coherent advices for treatment correction in T1D patients at rest and during challenging conditions in a standardized hospital environment. Ongoing investigations will assess the outcomes of prediction and advices on glucose control when provided online to patients.

Supported by: European Union FP7 Grant 216592

## 1030

### Fluorescence-based implantable glucose sensor with smartphone interface

T.K. Whitehurst, A.E. Colvin, A.D. DeHennis, J.C. Makous, M. Mortellaro, S. Rajaraman, J. Schaefer, D. Smith, S. Tankiewicz, O. Tymchyshyn, S. Walters, X. Wang;  
Sensors for Medicine and Science, Inc., Germantown, USA.

**Background and aims:** A fluorescence-based implantable glucose sensor has been developed. The small cylindrical sensor, which is approximately 3 mm in diameter and 14 mm long, has been designed to be inserted subcutaneously. A portion of the outer shell of the sensor includes a polymer hydrogel containing a proprietary indicator molecule that becomes fluorescent when it

binds glucose. The sensor includes a miniature fluorometer, with a tiny LED for excitation and multiple photodiodes for detection of the fluoresced signal. It also includes a highly accurate temperature sensor for compensation of the detected fluorescence. The glucose sensor has been designed to remain inserted for at least six months. The sensor is RF-powered, and an external body-worn reader is required to provide power to the sensor via a wireless inductive link. The reader also communicates with the sensor via a digital wireless inductive link to send commands to the sensor and to receive data from the sensor. The reader processes the sensed data to determine the sensor glucose value as well as the rate of change, and it is capable of storing up to 6 months of data. The reader includes a beeper and a vibration motor that are used to alert the patient, for example, when glucose passes a threshold value. The reader also includes a Bluetooth Low Energy link for communication with a smartphone as well as a USB port for charging and data exchange. Apps for the two most common smartphone platforms have been developed. The smartphone operates essentially as a user interface device for the reader, displaying data and providing user input, but it is not used to process sensor data or store data long-term. The smartphone app may be used to view sensor glucose data displayed graphically. The smartphone app also allows the user to enter data on daily events, for example, meals, insulin bolus administration, and exercise. All information entered by a patient is immediately transmitted to the reader.

**Materials and methods:** Two reader prototypes have been developed in support of pilot clinical studies. One is a wristwatch reader, designed for use with a sensor inserted subcutaneously in the dorsal wrist. The other is an armband reader, designed for use with a sensor inserted subcutaneously in the upper arm. The readers have each been programmed to read the implanted glucose sensor every two minutes. In a pilot study, 18 subjects were inserted bilaterally with a sensor in each arm. Each subject received at least one sensor in the wrist, while 7 subjects received the second sensor in the contralateral upper arm. The sensors remained inserted for 24 to 28 days, and subjects came to the clinic every three to six days to have the sensors read for 8 hours. Blood samples were also taken every 15 minutes and processed using a YSI Blood Glucose Analyzer. A single blood glucose value from the beginning of each clinic day was used to calibrate each sensor for each session, and the sensor glucose values were calculated prospectively for the session.

**Results:** The combined MARD for the 7 sensors inserted in the upper arm was 14.3%, and the MARD for the 20 sensors inserted in the wrist was 15.2%, using a single blood glucose value daily for calibration and prospective calculation of the sensor glucose values.

**Conclusion:** Clinical data from a 28-day pilot study of an implantable fluorescence-based glucose sensor has demonstrated the feasibility of the sensor.

## 1031

### Skin autofluorescence is associated with microvascular complications in type 2 diabetes

M.M.A. Yuen<sup>1</sup>, W. Chow<sup>1</sup>, C.H.Y. Fong<sup>2</sup>, K.S.L. Lam<sup>2</sup>;

<sup>1</sup>Department of Medicine, Queen Mary Hospital, <sup>2</sup>Department of Medicine, University of Hong Kong, Hong Kong

**Background and aims:** Skin autofluorescence (AF) is a marker of advanced glycation endproduct (AGE) accumulation in the body. AGEs are molecules formed through the non-enzymatic reactions of reducing sugars with proteins, lipids and nucleic acids. They accumulate at a constant but slow rate in the normal body. With increased glucose availability in diabetes, their formation is accelerated, and their deleterious effects on proteins have been implicated in diabetic complications. Upon excitation at 370 nm, AGEs have an emission spectrum at 440 nm, which is quantifiable as skin AF. This cross-sectional study investigated the relationship between skin AF and microvascular complications in Chinese type 2 diabetic subjects.

**Material and methods:** Subjects were recruited from the diabetic complication assessment program of our hospital. Skin AF was measured over the volar surface of the forearms using the AGE Reader. Three measurements were taken over each forearm according to the manufacturer's instructions, and the skin AF, in arbitrary units (AU), was recorded as the average of the three measurements on each side. To adjust for the effect of skin pigmentation, which absorbs light and thus influences skin AF, the AGE Reader automatically compared skin reflectance measurements across the 300 to 420 nm range with those of a white Teflon block, which was assumed to have 100% reflectance. Anthropometric and biochemical data including body height and weight, blood pressure, fasting glucose and lipids and HbA1c were collected. Statistical analysis was performed with SPSS 19.0.

**Results:** Skin AF was measured in 322 Chinese type 2 diabetic subjects (192 male, 130 female, 63.9±11.0 years), of which 229 had one or more microvascular complication(s). The median DM duration was 10 years (interquartile range 6–15 years) and the mean HbA1c was 7.84±1.35%. Skin AF correlated positively with age ( $r=0.350$ ,  $p<0.001$ ), DM duration ( $r=0.256$ ,  $p<0.001$ ), serum creatinine ( $r=0.280$ ,  $p<0.001$ ) and smoking pack-years ( $r=0.287$ ,  $p=0.006$ ), but not HbA1c ( $p=0.306$ ). There was no gender difference in skin AF ( $p=0.73$ ). Subjects with any microvascular complication had higher skin AF than those without ( $2.41\pm0.48$  AU vs.  $2.17\pm0.37$  AU,  $p<0.001$ ). Skin AF was higher in subjects with retinopathy ( $2.41\pm0.46$  AU vs.  $2.26\pm0.45$  AU,  $p=0.006$ ), nephropathy ( $2.49\pm0.49$  AU vs.  $2.20\pm0.39$  AU,  $p<0.001$ ) and neuropathy ( $2.56\pm0.51$  AU vs.  $2.27\pm0.42$  AU,  $p<0.001$ ) compared to those without the respective complication. Skin AF was independently associated with nephropathy (OR for one AU increase in AF 2.65 [1.42–4.95];  $p=0.002$ ) and neuropathy (OR for one AU increase in AF 2.28 [1.15–4.54];  $p=0.019$ ) after adjusting for gender, age, smoking status and DM duration. The optimal skin AF cut-off value for having any microvascular complication, nephropathy and neuropathy were 2.263 AU (sensitivity 59.8%, specificity 63.6%), 2.263 AU (sensitivity 68.8%, specificity 62.0%) and 2.307 (sensitivity 70.1%, specificity 57.6%) respectively on ROC analysis.

**Conclusion:** Skin AF was associated with diabetic complications, in particular nephropathy and neuropathy, in Chinese type 2 diabetic subjects. The AGE Reader might serve as a simple and non-invasive method to evaluate the risk of diabetic microvascular complication.

Clinical Trial Registration Number: HKCTR-1153

## 1032

### Local heating at the insulin injection site by the use of the InsuPad is able to reduce post-prandial glucose excursion in daily life

N. Hermanns, B. Kulzer, T. Haak;

Research Institute of Diabetes Academy Mergentheim (FIDAM), Bad Mergentheim, Germany.

**Background and aims:** The insulin action profiles of subcutaneously injected short acting insulin analogues are still slow compared to physiologically released human insulin. Thus, postprandial glucose excursions cannot be avoided. InsuPad is a medical device designed to accelerate insulin delivery rate by applying local heat at the insulin injection site. Whenever an insulin injection is given, the skin surface temperature is heated up locally to 38.5 °C for 30 minutes. This pilot-study examines the impact of the InsuPad use on postprandial glucose excursions after breakfast and dinner in daily life conditions.

**Materials and methods:** Insulin resistant diabetic patients were instructed to use the InsuPad when injecting bolus insulin prior to breakfast and dinner for one month and to measure their blood glucose at least five times per day (pre- and post-breakfast, pre-lunch and pre- and post-dinner). In the other study phase patients were instructed to maintain the same blood glucose measurement schedule for one month, without using the InsuPad. The order of the study phases was randomized. All blood glucose data were transmitted to a central computer using the DIASEND System. A valid pre-post-prandial measurement time difference was 75–135 minutes. An ANOVA, controlling for order of study phase, patient and meal (breakfast vs. dinner) was used to analyze the effect of the InsuPad on postprandial glucose excursions. In this study 10 diabetic patients took part (30% type 1 diabetes, age:  $51.5\pm7.7$  yrs., diabetes duration:  $15.7\pm7.7$  yrs.; HbA1c:  $8.2\pm0.9\%$ ; bolus insulin dose:  $51.7\pm22.2$  insulin units per day; total insulin dose 0.92 insulin units per kg).

**Results:** Preprandial blood glucose levels were similar in the phase with ( $151.7\pm46.8$  mg/dl) and without the InsuPad ( $148.5\pm38.6$  mg/dl,  $p=.385$ ). Postprandial glucose decreased by  $0.05\pm59.1$  mg/dl, if InsuPad was used, whereas the postprandial glucose levels increased by  $11.3\pm56.3$  mg/dl if the InsuPad was not used ( $p=.011$ ). The number (283 vs. 257) and proportion of valid measurements (65.5% vs. 64.4%,  $p=.740$ ) in the phase with and without InsuPad were highly comparable. The time differences between pre- and post-prandial glucose measurements were very similar in both study phases ( $102.7\pm19.0$  vs.  $102.3\pm15.6$  minutes with and without InsuPad,  $p=.777$ ). The overall glycaemic control (mean total blood glucose values) was significantly lower when using the InsuPad compared to the no-use-phase ( $149.7\pm54.5$  mg/dl vs.  $158.7\pm57.7$  mg/dl;  $p=.016$ ). The percentage of hypoglycaemic ( $<60$  mg/dl) or hyperglycaemic values ( $>300$  mg/dl) was slightly decreased when using the InsuPad, but the difference was not statistically significant (% hypoglycaemic values 1.5% vs. 1.8%,  $p=.496$ ; % hyperglycaemic values 1.6% vs. 2.1%,  $p=.250$ ).

**Conclusion:** This pilot-study indicates that local heating of the insulin injection site in insulin resistant diabetic patients by using the InsuPad is able to



reduce post-prandial blood glucose excursions as well as mean daily glucose values significantly in daily life conditions. Safety parameters like the prevalence of hypoglycaemic and hyperglycaemic glucose measurements were not affected by the use of InsuPad.

Supported by: InsuLine Medical Ltd

## 1033

### Phenomenology of free-living activities: quantifying what, when, and how much using new recognition methods based on triaxial accelerometry

C. Simon<sup>1,2</sup>, F. Gris<sup>3</sup>, J. Dugas<sup>1</sup>, T. Bastian<sup>1</sup>, A. Maire<sup>1</sup>, C. Villars<sup>1</sup>, M. Bourdin<sup>2</sup>, S. Blanc<sup>4</sup>, Y. Caritu<sup>5</sup>, E. Perrin<sup>5</sup>, P. Jallon<sup>3</sup>; <sup>1</sup>INSERM U1060, Lyon, <sup>2</sup>University of Lyon, <sup>3</sup>CEA-LETI, Grenoble, <sup>4</sup>IPHC, Strasbourg, <sup>5</sup>MOVEA, Grenoble, France.

**Background and aims:** Physical activity (PA) energy expenditure (EE) (PAEE) is a critical factor to consider for the prevention and treatment of diabetes and obesity. However, reference methods to measure PAEE are not easy to use routinely in free-living conditions. Motion sensors are being increasingly used as alternatives, but the posture and the nature of movements involved in different types of PA strongly affect the relationship between accelerometry data and PAEE. In particular, EE related to low intensity PA, the main activities in every-day life, is difficult to measure. Furthermore, in free-living conditions, the position of the device can hardly stay exactly the same at all times, which further affects PAEE estimates. This study aimed at testing the possibility to overcome these issues with an original algorithm using a new motion sensor coupling triaxial accelerometer and magnetometer.

**Materials and methods:** *Data acquisition:* 63 subjects aged 19 to 55 performed a set of standardized PA in the lab. Each subject was equipped with triaxial accelerometers (the new sensor and an Actigraph GT3X<sup>TM</sup>), and a combined accelerometer and heart-rate monitoring device (Actiheart<sup>TM</sup>). Indirect calorimetry (SERVOPRO 4100, Servomex, UK) provided simultaneous reference EE measurements. 29 subjects had a normal BMI, 17 were overweight and 17 obese. *Classification Algorithm:* Data from the new device were used to develop a classification algorithm. First, orientation of the sensor was estimated by advanced accelerometer signal processing. Then, a machine learning approach based on hidden Markov models was applied on the processed data to identify 6 types of PA. Several classifiers were tested, and their performance addressed with cross-validation procedures providing a good detection rate criterion (GDR = 1 if all identification events are correct). *PAEE predictions:* Accelerometer values were converted into counts on 2 axes and, for each group of PA, a predictive model of EE created using the indirect calorimetry data and classical regression methods. EE predictions for each identified PA were confronted with results from other devices, and from indirect-calorimetry, using MSE criteria. *Validation:* Validation in free-living conditions is still in progress. The recognition algorithm is tested by following 20 subjects performing different types of PA in a real-life setting over 3.5h. The quality of PAEE prediction models is tested with 120 subjects carrying all devices for 2 weeks. For 60 subjects, doubly labeled water will provide reference values of PAEE over these 2 weeks.

**Results:** *Classification Algorithm:* Of all classifiers tested, Naïve Bayes models identifying 6 groups of PA gave the best recognition results. The GDR for each activity was: 0.93 for lying, 0.93 for slouching, 0.69 for sitting, 0.87 for standing, 0.73 for walking, 0.63 for active walking; overall mean: 0.79. *PAEE Predictions:* The new approach predicted PAEE better than the other triaxial accelerometer, and better than, or as well as, the Actiheart<sup>TM</sup> built-in group algorithms.

**Conclusion:** Accelerometer coupled to magnetometer and performing recognition algorithms can overcome the main limitations of currently available tools in the measure of PAEE, and offer interesting tools for the follow-up of diabetic and/or obese patients.

Clinical Trial Registration Number: 2011-A01564-37

Supported by: French Nat. Res. Agency TECSCAN

## PS 087 Blood glucose self monitoring

## 1034

### Structured SMBG intervention in patients with non insulin treated type 2 diabetes: the PRISMA study

F. Giorgino<sup>1</sup>, M. Scavini<sup>2</sup>, A. Ceriello<sup>3</sup>, D. Cucinotta<sup>4</sup>, A. Tiengo<sup>5</sup>, E. Bonizzoni<sup>6</sup>, R. Marino<sup>7</sup>, E. Bosi<sup>2</sup>;

<sup>1</sup>Endocrinology & Metabolic Diseases, University of Bari, Italy, <sup>2</sup>Internal Medicine, San Raffaele Scientific Institute, Milan, Italy, <sup>3</sup>CIBERDEM, Centro de Investigacion Biomedica en Red de Diabetes y Enfermedades Metabolicas Asociadas, Barcelona, Spain, <sup>4</sup>Internal Medicine, Policlinico Universitario Gaetano Martino, Messina, Italy, <sup>5</sup>Clinical and Experimental Medicine, University of Padua, Italy, <sup>6</sup>Medical Statistics and Biometry GA Maccacaro, University of Milan, Italy, <sup>7</sup>Diabetes Care, Roche Diagnostics, Monza, Italy.

**Background and aims:** Recent studies on structured testing algorithms for self monitoring of blood glucose (SMBG) in patients with non insulin treated (NIT) type 2 diabetes (T2D) have shown improvements in HbA1c and other diabetes outcomes. However, not all open questions have been answered so far. Aim of this study was to test whether structured SMBG (intensive structured monitoring, ISM) compared to discretionary, unstructured SMBG (active control, AC) improves HbA1c through the optimization of diabetes treatment in patients with NIT T2D.

**Materials and methods:** The PRISMA study is a 12-month, prospective, multicenter, open, parallel groups, randomized trial to evaluate the added value of an intensive, structured SMBG regimen in NIT T2D patients. All patients participated in a standard education program and were then randomized to either ISM (4 SMBG measurements 3 days/week, including fasting, pre-prandial, 2 h post-prandial and post-absorptive) or AC (50 measurements over 3 months). Patients randomized to ISM were trained to respond to SMBG values outside targets by lifestyle changes. During a 12-month follow-up, patients were assessed quarterly with diabetes medications prescribed based on HbA1c and SMBG to target fasting and/or post-prandial hyperglycemia and to avoid hypoglycemia in the ISM group or on HbA1c only in the AC group. Two primary endpoints were tested in the following hierarchical order: 1) the change in HbA1c levels from baseline to month 12; and 2) the proportion of participants reaching or maintaining the risk targets [Low Blood Glucose Index (LBGI)  $\leq 2.5$  together with High Blood Glucose Index (HBGI)  $\leq 5$ ] from baseline to month 12.

**Results:** We recruited 1,024 patients (age  $60 \pm 8.5$  yrs, 40% females, BMI  $30.5 \pm 5.4$ ) with NIT T2D (duration  $6.2 \pm 3.8$  yrs; baseline HbA1c  $7.4 \pm 0.7\%$ ) in 39 diabetes centers in Italy, randomized to ISM (n=501) or AC (n=523). HbA1c decreased significantly more in ISM than AC patients [difference in HbA1c at month 12:  $-0.12\%$  (95% CI  $-0.210$  to  $-0.024$ ),  $p=0.013$ , ITT population, n=949;  $-0.21\%$  ( $-0.331$  to  $-0.089$ ),  $p<0.001$ , PP population, n=553]. Patients reaching or maintaining the LBGI and HBGI risk targets in the ISM compared to the AC group were 84.2% vs 80.2% in ITT ( $p=0.122$ ), and 90.0% vs 82.5% in PP ( $p=0.038$ ) populations, respectively. Changes in prescription of diabetes medications occurred more often in the ISM than AC patients (54.3% vs 45.7%, respectively;  $p<0.001$ ). BMI decreased more in the ISM than in the AC group [difference in BMI at month 12:  $-0.16$  (95% CI  $-0.343$  to  $0.013$ ),  $p=0.07$ , ITT population;  $-0.29$  ( $-0.528$  to  $0.061$ ),  $p=0.014$ , PP population]. Two severe hypoglycemia occurred in one patient in the AC group.

**Conclusion:** In patients with NIT T2D and close-to-target HbA1c the use of structured SMBG to optimize medications and lifestyle changes improved HbA1c, without increasing BMI and with no risk of severe hypoglycemia.

Clinical Trial Registration Number: NCT00643474

Supported by: Roche Diagnostics Italy

## 1035

### More frequent SMBG is associated with more frequent insulin boluses and lower HbA<sub>1c</sub>: baseline results from the ABACUS

R. Ziegler<sup>1</sup>, D.A. Cavan<sup>2</sup>, I. Cranston<sup>3</sup>, K. Barnard<sup>4</sup>, K. Barnard<sup>4</sup>, J. Ryder<sup>2</sup>, C. Vogel<sup>5</sup>, C.G. Parkin<sup>6</sup>, W. Koehler<sup>7</sup>, I. Vesper<sup>8</sup>, B. Petersen<sup>8</sup>, M. Schweitzer<sup>8</sup>, R.S. Wagner<sup>9</sup>;

<sup>1</sup>Diabetes Clinic for Children and Adolescents, Muenster, Germany, <sup>2</sup>Royal Bournemouth Hospital, UK, <sup>3</sup>Queen Alexandra Hospital, Portsmouth, UK, <sup>4</sup>University of Southampton, UK, <sup>5</sup>Internistisches Facharztzentrum, Langen, Germany, <sup>6</sup>CGParkin Communications, Inc., Boulder City, USA, <sup>7</sup>baseline statistics GmbH, Mannheim, Germany, <sup>8</sup>Roche Diagnostics GmbH, Mannheim, Germany, <sup>9</sup>Roche Diagnostics, Inc., Indianapolis, USA.

**Background and aims:** Self-monitoring of blood glucose (SMBG) is generally considered to be essential to the management of patients with type 1 (T1DM) and type 2 (T2DM) diabetes on multiple daily insulin injection (MDI) therapy. Although previous studies have shown an association between frequent SMBG and glycaemic control, we examined baseline data from a large clinical trial to determine if SMBG frequency actually correlated with better clinical outcomes.

**Materials and methods:** Baseline data were drawn from 219 T1DM (n=202) and T2DM (n=17) subjects enrolled in the Automated Bolus Advisor Control and Usability Study (ABACUS), a large, prospective, randomized, multi-national study of poorly controlled (HbA<sub>1c</sub> ≥ 7.5% / 58 mmol/l), MDI-treated patients. Subjects had a mean (SD) age 42.5 (14.1) years, BMI 26.4 (4.4), and HbA<sub>1c</sub> 8.85% (1.15). Demographic data, daily insulin bolus dose, HbA<sub>1c</sub> values, and self-reported SMBG frequency were correlated.

**Results:** Mean (SD) frequency of self-reported SMBG was 4.29 (1.51) tests/day. Frequency of SMBG was not significantly different between T1DM and T2DM subjects: 4.34 (1.51) vs. 3.76 (1.35) times per day; p = ns. SMBG frequency was similar in women and men: 4.25 (1.24) vs. 4.33 (1.70) times per day; p = ns. Mean (SD) frequency of SMBG was lower in subjects <30 years of age than older subjects: 3.83 (1.72) vs. 4.44 (1.41); p < 0.01. SMBG frequency did not correlate with education level, time from diagnosis to start of MDI, or length of time on MDI. Within the cohort, mean (SD) frequency of self-reported boluses was 3.54 (0.93) per day, with no differences according to type of diabetes, or gender. There was an association between frequency of SMBG and of boluses (r = 0.44) but not to total daily insulin bolus dose. There was a significant correlation with HbA<sub>1c</sub> (of r = -0.30), suggesting that more frequent SMBG is associated with lower HbA<sub>1c</sub>, independent of the type of diabetes.

**Conclusion:** These data suggest that patients treated with MDI therapy perform SMBG on average of 4-5 times daily independent of the type of diabetes, gender, or education level; however, younger patients tended to test less than older patients. We conclude that greater SMBG frequency was associated with more frequent insulin boluses and lower HbA<sub>1c</sub>. Frequent SMBG, when utilized to actively manage MDI therapy, may lead to more efficacious therapy and improved diabetes control. This will be evaluated in the ABACUS.

Clinical Trial Registration Number: NCT01460446

Supported by: Roche Diagnostics, Inc.

## 1036

### Information management improves medical outcome and supports therapy decision in diabetes care: results from the multicenter observational VISION study

J. Weissmann<sup>1</sup>, A. Müller<sup>1</sup>, K. Pralle<sup>2</sup>, H.-J. Ruessmann<sup>3</sup>, B. Gregersen<sup>4</sup>, D. Messinger<sup>5</sup>, I. Amann-Zalan<sup>1</sup>;

<sup>1</sup>Roche Diagnostics Deutschland GmbH, Mannheim, Germany, <sup>2</sup>Diabeteszentrum am Sophie-Charlotte-Platz, Berlin, Germany, <sup>3</sup>Diabeteszentrum & Hausarztpraxis, Dinslaken Germany, <sup>4</sup>General Practice, Osted, Denmark, <sup>5</sup>Biometrics Department, IST GmbH, Mannheim, Germany.

**Background and aims:** Many people with diabetes do not achieve optimal glycaemic control despite executing self-monitoring of blood glucose (SMBG). The challenge of analyzing and interpreting the large amount of complex SMBG data has been suggested to impede a better performance. Computerized data management offers a method to facilitate this data process procedure. The prospective VISION study aimed to assess the effectiveness of information management (IM) with the Accu-Chek® Smart Pix system, a tool for uploading, analyzing and reporting blood glucose (BG) data, in usual outpatient diabetes care. Particularly the effects on process quality of diabetes

management as well as on patients' attitude to diabetes treatment were examined.

**Materials and methods:** 914 adults with type 1 and type 2 diabetes mellitus, respectively (T1DM, n=248 / T2DM, n=666 - thereof 88% insulin treated) with poor glycaemic control (HbA<sub>1c</sub> ≥ 7.5%) from 123 primary care centers in Germany and Denmark were enrolled and monitored for about 7 months. BG data were analyzed, put into graphs and tables and summed up in reports by the Accu-Chek Smart Pix system. To evaluate the everyday benefit of using these reports as basis for therapy decision and patient communication, HbA<sub>1c</sub> status and physician's opinion on the diabetes management process were assessed via a questionnaire before (baseline) and approx. 3 and 6 months after IM integration into the practice workflow. Particularly therapy adjustments and the role of the reports in this matter were documented and analyzed. According to the observational nature of the study, descriptive and explorative statistical methods were used to analyze the collected data after stratification by diabetes type (T1DM/T2DM). The degree of significance of the test results was set at α<0.05.

**Results:** Within approx. 3 and 6 months of IM usage, HbA<sub>1c</sub> significantly (p<0.0001) dropped on average by -0.61%/-0.64% in T1DM and by -0.88%/-0.93% in T2DM from baseline (T1DM: 8.48% / T2DM: 8.66%). This clinically relevant effect was accompanied by a distinct and significant (p<0.01) improvement in patient therapy understanding and compliance as well as by an optimization of the therapy decision process (e.g. due to time savings) and of the dialog with patients, as assessed by physicians. The current diabetes therapy of 56.9% T1DM and of 64.0% T2DM patients, was adjusted in the course of the study. Mostly, adaptations referred to the insulin therapy (up to 39.9%) and to lifestyle modifications (up to 20.6%). In the majority of these cases (T1DM: ≥65% / T2DM: ≥81%) physicians stated that their decision was triggered by information provided by the Accu-Chek Smart Pix reports used.

**Conclusion:** Integration of IM in usual outpatient diabetes care improved glycaemic status significantly and lasting - in T2DM even more than in T1DM. Also enhancement of process and outcome quality - concerning therapy decision, patients' communication and attitude to diabetes treatment - was stated by physicians. This suggests that computerized data management can provide valuable support to physicians in analyzing, interpreting and communicating complex SMBG data and patterns.

## 1037

### Evaluation of blood glucose monitoring system pattern alert messages in a home setting: patient reported insights

M. Grady<sup>1</sup>, D. Campbell<sup>1</sup>, K. MacLeod<sup>2</sup>, A. Srinivasan<sup>2</sup>, P. Raja<sup>2</sup>;

<sup>1</sup>LifeScan Scotland Ltd, Inverness, UK, <sup>2</sup>LifeScan Inc., Milpitas, USA.

**Background and aims:** Blood glucose monitoring systems (BGMSs) that contain on-meter pattern recognition software are intended to advise users via timely on screen messages about recent high and low glucose patterns. This enables users to gain insights into these events and to consider making changes in diabetes management or lifestyle. However, there are minimal data on user perception of these messages or user awareness of why specific patterns were generated.

**Materials and methods:** A clinical study was conducted to evaluate user perceptions of patterns generated during 4 weeks home use of the OneTouch® Verio Pro BGMS by 101 individuals with Type 1 or Type 2 diabetes who self-adjust insulin. Participants provided feedback (using pre-defined reason codes or free-text; Table) in home diaries about the patterns they received during routine testing.

**Results:** In total, 987 coded reasons and 199 free-text reasons were recorded (Table). Participant responses were further segregated with respect to before meal high, fasting high or low pattern alerts. Categorizing the coded reasons for such patterns from these participant responses highlighted consistent themes. 37% of before meal high alerts and 46% of fasting high alerts resulted from either snacking or from insufficient basal insulin, respectively. Whereas low alerts (41%) were thought to result from activity of some kind. Categorizing participant free-text reasons also highlighted consistent themes with 21% of before meal high alerts and 21% of fasting high alerts resulting from either food / snacking or from insufficient basal insulin/ wrong carbohydrate ratio, respectively. Whereas low alerts (29%) were thought to result from insufficient food.

**Conclusion:** This study provides insights on how BGMS users interpret on-meter pattern alert messages and identify opportunities for improving glycaemic control. The users' observations may also facilitate conversations with HCP's.

## Participant Responses to Specific Pattern Alerts

Alert Type	n	Coded Reasons
Before meal high	557	Snacking (37%), Miscounting carbohydrates (15%), Insufficient mealtime insulin (14%)
Fasting high	209	Insufficient basal insulin (46%), Snacking at night (32%), Rebound hyperglycemia (21%)
Low	221	Activity (41%), Too much insulin (25%), Miscounting carbohydrates (16%)
<b>Free-text Reasons</b>		
Before meal high	105	Food or snacking (21%), Don't know (14%), Rebound hypoglycemia (13%)
Fasting high	70	Insufficient insulin or wrong ratio to carbohydrates (21%), Food or snack at bedtime (21%), Illness or infection (14%)
Low	24	Insufficient food (29%), Don't know (25%), Too much insulin / over- correcting high glucose (12%)

## 1038

**Accuracy of a blood glucose monitoring system at hypoglycaemic glucose levels: analysis of seven clinical studies**

J. Diago Cabezudo<sup>1</sup>, S. Bellary<sup>2</sup>, H. Cameron<sup>3</sup>, K. MacLeod<sup>3</sup>, J. Ellison<sup>3</sup>, P. Raja<sup>3</sup>;

<sup>1</sup>LifeScan, Johnson & Johnson S.A., Madrid, Spain, <sup>2</sup>Aston University, Birmingham, UK, <sup>3</sup>LifeScan Inc., Milpitas, USA.

**Background and aims:** For individuals with diabetes detection of hypoglycemia is crucial, and blood glucose monitoring systems (BGMs) must therefore be accurate in hypoglycemic ranges. Collecting sufficient data at low blood glucose (BG) levels is difficult; which may explain why evidence of BGMS performance during hypoglycemia is lacking. This analysis evaluated the accuracy of 5 performance-equivalent BGMs using OneTouch<sup>®</sup> Verio<sup>®</sup> test strips at BG levels of < 3.9 mmol/L (70 mg/dL) in 7 separate clinical studies.

**Materials and methods:** The studies were conducted between June 2009 and June 2011. BG testing was performed by trained staff in 2 clinical centers in the UK. All studies were conducted in accordance with standard ISO 15197:2003 testing guidelines. Each study included 100 participants with diabetes. For each participant, duplicate BGMS tests were performed using 3-4 test strip lots per study. BG reference values were obtained using a YSI 2300 STAT Glucose Analyzer before and after BGMS testing.

**Results:** Of a total of 5,400 measurements, 674 (12.5%) were at a BG level of < 3.9 mmol/L (70 mg/dL). The number and percentage of results within  $\pm 0.83$  mmol/L (15 mg/dL) and  $\pm 0.56$  mmol/L (10 mg/dL) were calculated at BG levels < 3.9 mmol/L (70 mg/dL), < 3.3 mmol/L (60 mg/dL), and < 2.8 mmol/L (50 mg/dL). Accuracy of the OneTouch Verio test strips at hypoglycemic blood glucose levels are shown in the Table.

**Conclusion:** Compared with analysis based on a single study, using data from 7 studies, 5 BGMs, and 15 different test strip lots provides a robust depiction of test strip accuracy at BG levels < 3.9 mmol/L (70 mg/dL). The results indicate that OneTouch Verio test strips are highly accurate in the hypoglycemic range. Table. OneTouch Verio accuracy at BG levels < 3.9 mmol/L (70 mg/dL).

	BG values		
	< 3.9 mmol/L (70 mg/dL)	< 3.3 mmol/L (60 mg/dL)	< 2.8 mmol/L (50 mg/dL)
*Within $\pm 0.83$ mmol/L (15 mg/dL), % (n/N)	100.0 (674/674)	100.0 (358/358)	100 (270/270)
Within $\pm 0.56$ mmol/L (10 mg/dL), % (n/N)	98.8 (666/674)	100.0 (358/358)	100 (270/270)

\*ISO 15197:2003 accuracy limits for BG levels < 4.2 mmol/L (75 mg/dL).

## 1039

**Accuracy of self monitoring blood glucose systems in different ranges of blood glucose**

F. Kulozik, I. Platten, C. Hasslacher;  
Diabetesinstitut Heidelberg, Germany.

**Background and aims:** The accuracy of self monitoring blood glucose (SMBG) systems is usually assessed according to the Guidelines of the International Organization for Standardization (ISO). Currently a revision of the relevant standard ISO 15197 presumably resulting in more stringent criteria is in progress. To meet those criteria at least 95% of the SMBG values may not deviate more than 15 mg/dl from the reference values at BG concentrations of < 100 mg/dl and more than 15% at BG concentrations  $\geq 100$  mg/dl respectively. Also a differentiation of BG levels in more than those two ranges is being discussed. Therefore the aim of the present study was to evaluate the accuracy of customary SMBG systems according to various BG ranges.

**Materials and methods:** We evaluated the accuracy of 20 customary SMBG systems according to the currently discussed revised version of ISO 15197, differentiating BG ranges of < 100 mg/dl, 100-149 mg/dl, 150-199 mg/dl, 200-249 mg/dl, and  $\geq 249$  mg/dl. Therefore an increase and decrease of BG values in the range of approximately 50 - 300 mg/dl were induced by administration of carbohydrate intake and / or insulin application in insulin dependent diabetics. Capillary blood samples were taken from the fingertip for laboratory glucose determination by means of Hitado Super GL (glucose oxidase method) as reference and BG values were simultaneously measured in capillary blood from the same location using a SMBG device. The following commercially available glucose meters were tested: AccuChek<sup>®</sup> Compact, AccuChek<sup>®</sup> Mobile, AccuChek<sup>®</sup> Aviva Nano (Roche Diagnostics), BG Star<sup>®</sup> (AgaMatrix Inc), Breeze<sup>®</sup>, Contour<sup>®</sup> Plasma, Contour<sup>®</sup> USB (Bayer Health Care), Fine Touch<sup>®</sup> (Terumo Corp.), Freestyle Lite<sup>®</sup> (Abbott Diabetes Care), GL 40<sup>®</sup> (Beurer Medical), Glucomen LX<sup>®</sup> (Menarini Diagnostics), GlucoSmart<sup>®</sup> Swing (MSP Bodmann), MyLife Pura<sup>®</sup> (Ypsomed AG), Omnitest3<sup>®</sup> (B. Braun), One Touch Ultra<sup>®</sup>, One Touch Verio<sup>®</sup>, One Touch Vita<sup>®</sup> (LifeScan Inc), smartLAB mini<sup>®</sup>, smartLAB sprint<sup>®</sup> (HMM Diagnostics), Wellion Calla<sup>®</sup> (MedTrust). For each SMBG system 200 - 300 pairs of values were obtained.

**Results:** The analyzed SMBG systems showed significant differences concerning the accuracy regarding to the mentioned BG ranges varying from only 70% to often 100% of the values within the ISO criteria. We could demonstrate a high level of accuracy for 7 SMBG systems, i.e. 95% of the values met the ISO standard in at least 4 BG ranges (AccuChek<sup>®</sup> Compact, AccuChek<sup>®</sup> Mobile, AccuChek<sup>®</sup> Aviva Nano, Freestyle Lite<sup>®</sup>, MyLife Pura<sup>®</sup>, One Touch Ultra<sup>®</sup>, One Touch Vita<sup>®</sup>). 9 glucose meters fulfilled the criteria in only 2 or even less BG ranges, the inaccuracy of those devices was found in the lower ranges of BG in particular.

**Conclusion:** The results of the present study proved that only 1/3 of the examined SMBG systems had adequate accuracy in at least 4 of the 5 chosen BG ranges. The glucose meters with insufficient accuracy showed their weakness particularly in the lower ranges of BG.

## 1040

**Inaccuracies found in common SMBG systems: clinical considerations for patients and providers**

R. Brazg<sup>1</sup>, L. Klaff<sup>1</sup>, C. Parkin<sup>2</sup>;

<sup>1</sup>Rainier Clinical Research Center, Renton, <sup>2</sup>CGParkin Communications, Inc., Boulder City, USA.

**Background and aims:** Self-monitoring of blood glucose (SMBG) is an important component of diabetes management. Because SMBG data are often used in clinical decision-making, it is critical that the data be accurate. Several variables (e.g. user technique) can impact the accuracy of SMBG results. In clinical practice the inherent accuracy of the SMBG system itself is often not considered. It is assumed that because all systems marketed in the US met the current international standard ISO 15197:2003 (ISO), they must be accurate. This assumption is not necessarily true; concerns about SMBG accuracy have prompted the development of new criteria for accuracy that require all systems to achieve a performance level whereby  $\geq 95\%$  of the individual glucose results fall within  $\pm 15$  mg/dL of the results of the manufacturer's measurement procedure at glucose concentrations <100 mg/dL and within 15% for values  $\geq 100$  mg/dL. We evaluated the accuracy of 7 currently marketed systems against the current and proposed accuracy criteria: ACCU-CHEK<sup>®</sup> Aviva Plus; Advocate<sup>™</sup> Redi-Code; Element<sup>™</sup>; Embrace<sup>™</sup>; Prodigy<sup>®</sup> Voice; TRUEbalance<sup>™</sup>; and WaveSense Presto<sup>™</sup>.



**Materials and methods:** Capillary blood samples were collected from 162 subjects by deep finger puncture. Results from the SMBG systems were compared to those obtained from each manufacturer's documented reference system, YSI or PCA-HK; 3 different strip lots from each SMBG system were tested with each subject, in duplicate.

**Results:** Evaluated using the current ISO standard ( $\geq 95\%$  within  $\pm 15$  mg/dl for values  $< 75$  mg/dl and  $\pm 20\%$  for values  $\geq 75$  mg/dl), only the ACCU-CHEK Aviva Plus, Element, and WaveSense Presto systems met accuracy criteria; however, only the ACCU-CHEK Aviva Plus met the newly proposed criteria in all 3 lots tested. (Table) The other 6 systems failed to meet the standard in at least 2 of the 3 lots tested, showing lot-to-lot variability, high/low bias and variations due to hematocrit.

**Conclusion:** Whilst only 3 of 7 systems tested met the current ISO accuracy standard, only 1 of 7 met the proposed accuracy criteria. Because SMBG data are frequently used to make therapeutic decisions, inaccurate glucose readings can potentially adversely impact clinical outcomes in people with diabetes. SMBG accuracy is particularly important in the elderly (who are often more susceptible to hypoglycemia) and those with diabetic nephropathy (who are often anemic). Although there are many factors that should be addressed through patient education and training, clinicians can reduce controllable variables, by prescribing accurate and validated SMBG systems. The proposed accuracy criteria should enhance patient safety by improving the accuracy of available SMBG systems. Table. Percentage and number of tests within range: Current ISO and Proposed ISO criteria

SMBG System (Reference Method)	ISO 15197			Proposed ISO 15197		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
	% (Tests)	% (Tests)	% (Tests)	% (Tests)	% (Tests)	% (Tests)
ACCU-CHEK Aviva Plus (PCA-HK) Group 1	100% (200/200)	99.5% (199/200)	99.5% (199/200)	100% (200/200)	99.0% (198/200)	98.5% (197/200)
Advocate Redi-Code (YSI)	96.5% (193/200)	95.0% (190/200)	88.5%* (177/200)	93.0%* (186/200)	86.5%* (173/200)	84.0%* (168/200)
Embrace (YSI)	95.0% (190/200)	93.0%* (186/200)	97.0% (194/200)	89.5%* (179/200)	87.0%* (174/200)	87.5%* (175/200)
TRUEbalance (YSI)	96.0% (192/200)	91.0%* (182/200)	97.5% (195/200)	87.0%* (174/200)	87.0%* (174/200)	93.5%* (187/200)
ACCU-CHEK Aviva Plus (PCA-HK) Group 2	99.0% (198/200)	99.0% (198/200)	99.0% (198/200)	97.0% (194/200)	97.5% (195/200)	98.5% (197/200)
WaveSense Presto (YSI)	95.0% (190/200)	95.0% (190/200)	97.5% (195/200)	87.0%* (174/200)	88.0%* (176/200)	85.0%* (170/200)
Element (YSI)	97.0% (194/200)	98.0% (196/200)	95.5% (191/200)	94.0%* (188/200)	93.5%* (187/200)	90.5%* (181/200)
Prodigy Voice (YSI)	88.5%* (177/200)	98.0% (196/200)	95.0% (190/200)	84.0%* (168/200)	96.5% (193/200)	93.0%* (186/200)

\* Failed to meet current or newly proposed accuracy criteria

Supported by: Roche Diagnostics, Inc.

## 1041

### Projection of long term health-economic benefits of continuous glucose monitoring (CGM) versus self monitoring of blood glucose in type 1 diabetes, a UK perspective

S. Roze<sup>1</sup>, P. Lynch<sup>2</sup>, M. Cook<sup>3</sup>

<sup>1</sup>HEVA, Lyon, France, <sup>2</sup>Medtronic International Trading Sarl, Tolochenaz, Switzerland, <sup>3</sup>Medtronic UK, Watford, UK.

**Background and aims:** The main objective of this study was to estimate the impact of Continuous Glucose Monitoring (CGM) compared to Self Monitoring of Blood Glucose (SMBG) on both health and economic outcomes of type 1 diabetes (T1DM) in the UK.

**Materials and methods:** The Core Diabetes Model (CDM) is an internet-based, highly validated, computer simulation model to determine the long-term health outcomes and economic consequences of diabetes interventions. This model was used to project the incidence of diabetes-related complications over a lifetime horizon, based on A) a recently published meta-analysis comparing CGM versus SMBG, with insulin delivery being the same in both arms and B) a real life observational study. The meta-analysis showed that for

the analysed cohort of T1DM with average baseline HbA1c of 10%, every day use of CGM led to a reduction of -1.49% versus -0.62% HbA1c, for CGM and SMBG respectively. The mean baseline age of the simulated cohort was 27 years, with a mean duration of diabetes of 13 years. The observational study recorded a reduction from 7.11 to 4.35 daily blood glucose tests respectively for SMBG and CGM groups. CGM effects also included a decrease in the annual rate of major hypoglycaemic events from 27.7 events per 100 patients' years for SMBG to 15. The quality of life was adjusted for a reduced fear of hypoglycaemic event in the CGM arm. Diabetes related complication costs were UK specific and taken from various published sources. Discount rates were applied on both clinical and economic outcomes respectively at a rate of 1.5% and 3.5% per annum. Sensitivity analyses were carried out on several key parameters.

**Results:** The incremental cost-effectiveness ratio (ICER) was 17,932 GBP (£) per Quality Adjusted Life Year gained (QALY). The improvement in discounted QALY was 1.9 years in favour of CGM. Undiscounted life expectancy was increased by 3.03 year for CGM versus SMBG. Additional CGM related costs were partially offset by the savings due to the reduction in diabetes related complications and the lower frequency of SMBG tests. Remaining extra costs due to CGM were on average 1,361£ per year. CGM usage compared to SMBG increased the mean time alive free from any complications by 2.87 years. In a one-way sensitivity analysis considering no effect on the rate of major hypoglycaemic event for CGM, the ICER was 23,067£/QALY. The one-way sensitivity analysis based on no reduction of fear of hypoglycaemic event due to CGM led to an ICER of 21,336£/QALY. By using an equal discount rate for both health and economic parameters of 3.5%, the ICER was 25,975 £/QALY.

**Conclusion:** Using a well-accepted simulation model in T1DM, projection of the improvement in HbA1c of CGM versus SMBG translated into cost-effective ratio, generally considered as very good value for money in the UK. Extensive sensitivity analysis on key drivers confirmed the robustness of results under a wide range of assumptions. This analysis did not include indirect costs, which would have led to even better health-economic results from a societal perspective.

Supported by: Medtronic

## PS 088 Continuous glucose monitoring

### 1042

#### Accuracy and acceptability of the 6-day Enlite continuous subcutaneous glucose sensor

S.W. Lee<sup>1</sup>, T. Bailey<sup>2</sup>, R. Brazg<sup>3</sup>, M. Christiansen<sup>4</sup>, A. Ahmann<sup>5</sup>, R. Henry<sup>6</sup>, S. Garg<sup>7</sup>, E. Watkins<sup>8</sup>, F.R. Kaufman<sup>1</sup>

<sup>1</sup>Medtronic, Inc., Northridge, <sup>2</sup>AMCR Clinic, Inc., Escondido, <sup>3</sup>Rainier Clinical Research Center, Renton, <sup>4</sup>Diablo Clinical Research, Walnut Creek, <sup>5</sup>Oregon Health and Science University, Portland, <sup>6</sup>University of California San Diego, <sup>7</sup>University of Colorado, Denver, Aurora, <sup>8</sup>Profil Institute for Clinical Research, Chula Vista, USA.

**Background and aims:** The Enlite subcutaneous glucose sensor was previously evaluated in adults and children and shown to be accurate for 6 days, durable, and acceptable to patients and parents/caregivers. We evaluated its accuracy using abdominal insertion sites at a wide range of glucose concentrations and at different rates of glucose concentration change.

**Materials and methods:** A pivotal 6-day trial in adults was conducted at 7 US investigational centers. Sensors were self-inserted and taped. Each patient wore 1 or 2 sensors on the abdomen and these sensors were calibrated 3–4 times per day throughout the study. Accuracy was evaluated vs frequently-sampled YSI plasma glucose values. The frequent sampling tests lasted for 12 hours on days 1, 3, and 6. Hypoglycemia (to  $\leq 75$  mg/dL) and hyperglycemia (to  $\geq 180$  mg/dL) were induced on day 1 (beginning immediately after initial calibration), day 3, and day 6. Accuracy was assessed on different days (1, 3, and 6) and at different glucose concentration ranges ( $\leq 75$  mg/dL, 75–180 mg/dL, and  $> 180$  mg/dL). Accuracy of calibration was also assessed at rapid, moderate, and slow absolute rates of change (|ROC|) ( $> 2$ , 1–2,  $< 1$  mg/dL/min, respectively). Patient satisfaction with Enlite was also evaluated with a 7-point Likert-type questionnaire.

**Results:** Adults with type 1 (N=65) or type 2 (N=25) diabetes (mean age 44, range 18–71) participated. The Table shows mean and median absolute relative differences (ARD) between sensor and YSI glucose concentrations by day and by glucose range. Mean self-reported survey responses were 5.9/7 for “ease of use,” 6/7 for “comfort,” 5.9/7 for “ease of insertion,” and 5.8/7 for “would recommend.” The overall mean (median) absolute relative differences (ARD) were 13.6% (10.1%). At rapid ROC the mean (median) ARD were 16.3% (12.9%), at moderate ROC were 12.9% (9.6%), and at slow ROC were 13.6% (10.1%). There were no device-related adverse events.

**Conclusion:** We conclude that the Enlite sensor is accurate, durable, comfortable, safe, and easy to use. It is accurate during periods of stable or changing glucose concentrations and met predefined success criteria for agreement with YSI values at all glucose ranges. Improvements in continuous glucose sensing should expedite development of semiautomated insulin delivery features in modern pumps.

Enlite sensor accuracy by mean and median absolute relative differences				
By Day	Overall	Day 1	Day 3	Day 6
Mean (Median) ARD	13.6% (10.1%)	15.9% (12.2%)	11.8% (9.1%)	13.2% (9.6%)
By Glucose Range	Overall	$< 75$ mg/dL	75–180 mg/dL	$> 180$ mg/dL
Mean (Median) ARD	13.6% (10.1%)	10.8* (8.5*)	12.7% (9.4%)	12.0% (9.0%)

\* , mean absolute differences (mg/dL)

### 1043

#### Comparison of three continuous glucose monitoring systems with six sensors per subject in parallel

M. Link<sup>1</sup>, G. Freckmann<sup>1</sup>, S. Pleus<sup>1</sup>, E. Zschorneck<sup>1</sup>, C. Haug<sup>1</sup>, H.-M. Klötzer<sup>2</sup>, E. Ramstetter<sup>3</sup>, W. Schmidt<sup>3</sup>, M. Schoemaker<sup>3</sup>

<sup>1</sup>Institute for Diabetes-Technology GmbH, Ulm, <sup>2</sup>HMKQ, Weinheim, <sup>3</sup>Roche Diagnostics GmbH, Mannheim, Germany.

**Background and aims:** The aim of this study was to compare 3 commercially available needle-type continuous glucose monitoring (CGM) systems

following the CLSI guideline POCT05 “Performance Metrics for Continuous Interstitial Glucose Monitoring” under daily life conditions.

**Materials and methods:** Twelve people with type 1 diabetes (age:  $47.7 \pm 6.9$  years (mean  $\pm$  standard deviation), HbA<sub>1c</sub>  $8.2 \pm 1.4$  %, duration of diabetes:  $23.7 \pm 9.9$  years) wore 6 sensors in parallel, two sensors each of the 3 following CGM systems: FreeStyle Navigator™, DexCom™ Seven® Plus 3<sup>rd</sup> Generation and Guardian® REAL-Time with Enlite Sensor. Each system was used for one sensor lifetime, which was 5 days for the FreeStyle Navigator™, 6 days for the Guardian® REAL-Time or 7 days for the DexCom™ Seven® Plus. CGM measurements were paired to capillary blood glucose (bG) measurements (approximately 30 per day and subject). Performance was characterized by mean absolute relative differences (MARD) between CGM and paired bG measurements and percentage absolute relative difference (pARD), which is the average of the absolute relative differences between paired CGM measurements of the first and the second sensor of each system.

**Results:** Average data reporting percentages ranged from 95.9 % (DexCom™ Seven® Plus) to 98.9 % (Guardian® REAL-Time) of the time worn. Capillary bG measurements ranged from 35 to 446 mg/dL. MARD and pARD are reported in the table (mean  $\pm$  standard deviation [range]). All systems could be worn for nearly 100 % of their estimated lifetime (FreeStyle Navigator™: 100.6 %, DexCom™ Seven® Plus: 98.3 % and Guardian® REAL-Time: 99.0 %) before the sensors had to be removed.

**Conclusion:** The 3 CGM systems tested showed good overall performance, with the FreeStyle Navigator™ achieving lower sensor-to-bG differences and sensor-to-sensor differences than the DexCom™ Seven® Plus 3<sup>rd</sup> generation and the Guardian® REAL-Time with Enlite Sensor.

Mean and percentage absolute relative differences for the CGM systems tested.

CGM system	MARD	pARD
FreeStyle FreeStyle Navigator™	12.4 $\pm$ 3.6 % [8.4 - 19.0 %] (n = 24)	10.1 $\pm$ 4.1 % [5.2 - 17.6 %] (n = 12)
DexCom™ Seven® Plus	16.7 $\pm$ 3.8 % [12.7 - 29.2 %] (n = 24)	15.4 $\pm$ 4.2 % [9.2 - 23.5 %] (n = 12)
Guardian® REAL-Time	16.4 $\pm$ 6.9 % [7.5 - 23.9 %] (n = 24)	18.1 $\pm$ 6.5 % [9.2 - 31.2 %] (n = 12)

Supported by: Roche Diagnostics GmbH

### 1044

#### HbA<sub>1c</sub> and mean glucose in insulin treated diabetes using the SevenPlus continuous glucose monitor (CGM): correlation and intra-patient consistency over time

N.B. Argento<sup>1</sup>, K. Nakamura<sup>2</sup>, R. Sala<sup>2</sup>

<sup>1</sup>Maryland Endocrine and Diabetes Center, Columbia, <sup>2</sup>Dexcom, Inc., San Diego, USA.

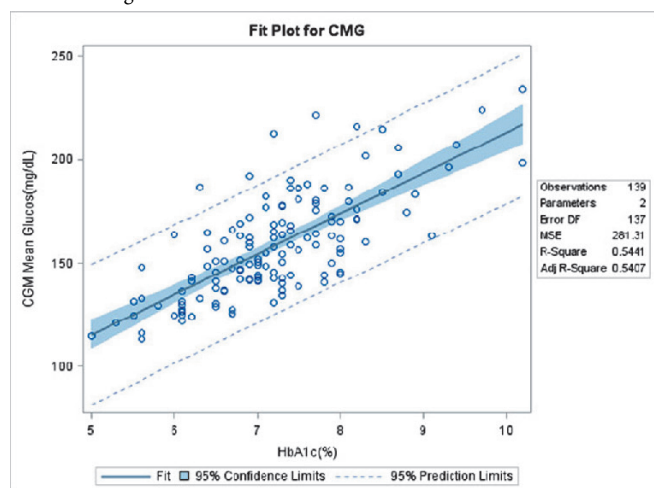
**Background and aims:** The relationship between HbA<sub>1c</sub> and mean glucose (MG) is controversial. An estimated HbA<sub>1c</sub> derived glucose (eAG) is said to be useful, but CGM data shows significant variation between the eAG and CGM measured MG (CMG). Little information is available on the consistency of the eAG-CMG relationship in an individual patient over time. We report the eAG-CMG relationship in a large group of insulin requiring diabetes patients using home CGM in a real world setting, and the consistency of this relationship in a subgroup of patients in whom more than one eAG-CMG value was available.

**Materials and methods:** Patients using the SevenPlus CGM at home have their CGM device downloaded for review as part of their routine clinic evaluation. Clinic data were reviewed, and an HbA<sub>1c</sub>-CMG data pair was included if there was more than 50% utilization of CGM in the 60 days prior to the HbA<sub>1c</sub>. A subgroup had between 2 to 6 data pairs available. A linear regression model for eAG-CMG was derived from the most recent data pair for each unique patient (n=139) and eAG-CMG consistency, measured by variance and range within a patient, were also evaluated in patients with multiple eAG-CMG pairs (n=91, mean values per patient 3.3).

**Results:** The mean age was 48.7 yr (18–75). Their diabetes duration was 26 yr (1–58). 61% were male. 91% had T1D and 9% had T2D. 80% were on CSII and 20% were on MDI treatment. The mean CGM utilization of the study population was 80% of maximal possible use. Figure. CMG (mgdl) =  $18 + 19.484 A_{1c}$ . The CMG for A<sub>1c</sub> 7.0% = 154.4 mg/dL (8.58 mmol/L). For pa-

tients with multiple eAG-CMG pairs, the residual between CMG and eAG ( $\text{CMG} - \text{eAG} / \text{eAG} \times 100$ ) was calculated for each eAG-CMG pair. Patients tended to trend consistently over multiple eAG-CMG determinations. For some patients, these differences have important clinical implications. For example, subj 1: CMG 192 mg/dL, eAG 156 mg/dL, residual 18.7%; CMG 212 mg/dL, eAG 158 mg/dL, residual 25.5%, difference of residuals 6.8%. Subj 2: CMG 145 mg/dL, eAG 174 mg/dL, residual -20.1%; CMG 142 mg/dL, eAG 160 mg/dL, residual -13.1%, difference 7.0%. The mean variance of residuals was 4.42%. The mean maximal difference between residuals was 7.3%. 74.8% of patients had a maximal difference between residuals of 10% or less (36.3% < 5.1%, 38.5% 5.1–10.0%, mean 5.04%), and 5.5% had a maximal difference >15% (mean=19.8%). For this small group with high variation of residuals, there was no significant difference in % CGM use, CMG or the CGM standard deviation of glucose values.

**Conclusion:** The SevenPlus CGM yields a similar eAG-CMG correlation to the JDRF CGM study. There is significant variation in the eAG and CMG in individual patients. Some subjects have striking differences between eAG and CMG, which has important clinical implications. The individual eAG-CMG relationship tends to trend consistently over time in most patients. Clinicians should consider modification of  $\text{HbA}_{1c}$  goals in patients with persistent significant divergence of eAG and CMG.



## 1045

### Performance of a microdialysis-based continuous glucose monitoring (CGM) system

E. Zijlstra<sup>1</sup>, T. Heise<sup>1</sup>, W. Künnecke<sup>2</sup>;

<sup>1</sup>Profil Institute for Metabolic Research, Neuss, <sup>2</sup>Trace Analytics GmbH, Braunschweig, Germany.

**Background and aims:** Combining intravenous microdialysis with on-line glucose analysis for CGM may prove to become an important development to achieve tight glycaemic control in hospitalised patients without blood loss, measurement delays or frequent manual interventions. In this study we evaluated the performance of a microdialysis-based CGM system for up to 48 hours use.

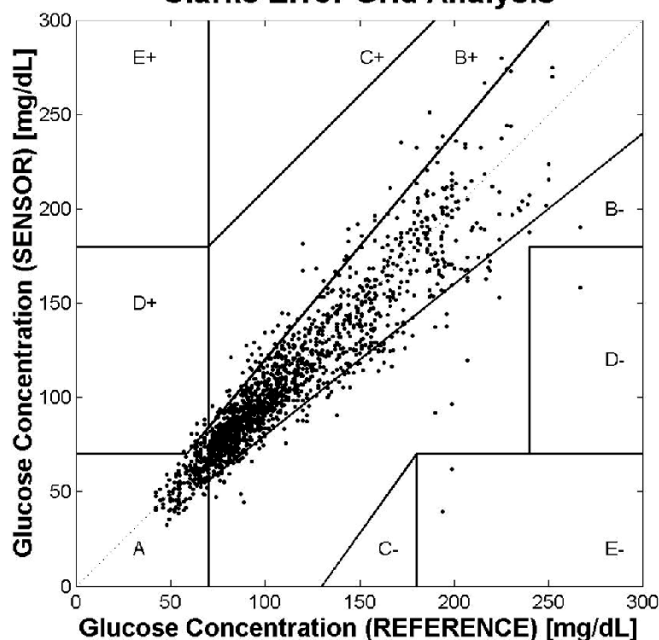
**Materials and methods:** Twenty-one healthy adult subjects were connected to 1 or 2 microdialysis-based CGM systems. Blood glucose was sampled automatically and without blood loss by means of microdialysis at least every 2 minutes. The dialysate containing the sampled glucose was analysed immediately by an on-line glucose sensor. The glucose sensor was calibrated prior to the start of an experiment and once more after 24 hours in experiments lasting for 48 hours. Reference blood samples were taken manually and analysed using a laboratory glucose analyser every 10–60 minutes. The subjects consumed meals or glucose was administered orally or intravenously to analyze the accuracy of the CGM system over a range of blood glucose concentrations.

**Results:** A total of 1796 paired sensor-reference data points were evaluated. Mean relative deviation was 9.4% overall, with only small deviations between hypoglycaemic (< 70 mg/dL), euglycaemic (70–180 mg/dL) and hyperglycaemic (> 180 mg/dL) glucose ranges (11.0%, 8.8% and 12.6% respectively). According to the current ISO15197 criteria 91.4% of data points were accurate. Good performance in the hypoglycaemic range is notoriously difficult, but

the first results with this new system are very encouraging: Below 75 mg/dL, 94.6% of the sensor values were accurate (within 15 mg/dL of the reference value). Clarke Error Grid analysis showed that 99.3% of the data points were located within the accurate and acceptable zones, Zone A & B. The remaining datapoints were located within Zone D (0.6%) and Zone E (0.1%).

**Conclusion:** This study shows that CGM using microdialysis in blood is feasible for up to 48 hours and provides reasonably accurate glucose results with only a once-daily calibration. Improved calibration and/or referencing strategies may increase the accuracy of the system further in future studies.

### Clarke Error Grid Analysis



## 1046

### Performance and reliability of the new Dexcom G4™ continuous glucose monitoring (CGM) System- Pivotal trial results

D. Price<sup>1</sup>, T. Bailey<sup>2</sup>, M. Christiansen<sup>3</sup>, E. Watkins<sup>4</sup>, D. Liljenquist<sup>5</sup>, K. Nakamura<sup>1</sup>;

<sup>1</sup>Dexcom, Inc, San Diego, <sup>2</sup>AMCR Institute, Inc, Escondido, <sup>3</sup>Diablo Clinical Research, Walnut Creek, <sup>4</sup>Profil, Chula Vista, <sup>5</sup>Rocky Mountain Diabetes and Osteoporosis Center, Idaho Falls, USA.

**Background and aims:** Since the first real-time CGM systems were approved, accuracy and reliability have been a concern for some patients and clinicians. We report on the results of a study for the new CGM which found improvements in performance and reliability compared with previous CGM systems due to changes in the sensor design, sensor materials, algorithm, and transmitter.

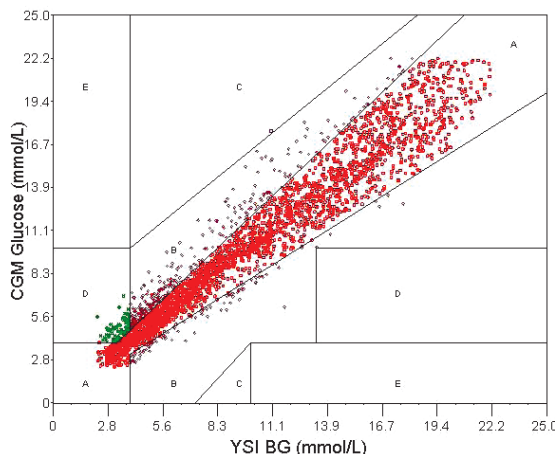
**Materials and methods:** The study enrolled 72 adult subjects (≥18 yrs, 83%T1DM, 17%T2DM) at 4 US centers. Subjects wore sensors for up to 7 days and 36 subjects wore two sensors concurrently to evaluate system precision. Patients calibrated their CGM using self-monitored blood glucose (SMBG) values obtained every 12 hours with a OneTouch Ultra 2 blood glucose meter. Subjects were in-clinic for 12 hours on days 1, 4 and 7 to have glucose deliberately raised and lowered so as to test the performance of the CGM over the entire measurement range (2.2 to 22 mmol/l) of the device. CGM readings were compared against Yellow Springs Instrument (YSI) reference plasma glucose measurements acquired from venous samples every 15 minutes.

**Results:** There were a total of 9,093 temporally matched points between the new CGM and YSI reference value. The global accuracy of the system was 13.2 % Mean ARD with a Median ARD of 9.5%. 80% of all points were in the Clarke Error Grid (CEG) A zone. CGM accuracy improved over the seven days of sensor wear. The Mean ARD on day 1 was 16.6% compared with 11.3% on day 4 and 11.8% on day 7. 85% of percent of all CGM glucose results obtained in-clinic on days 4 and 7 were in the CEG A zone (see figure), approaching the accuracy of BG meters during home use. Accuracy in the hypoglycemic region (2.2–4.4 mmol/l) was 0.7 mmol/l Mean Absolute



Difference and 83% of the data was within 1.1 mmol/l of the YSI values. 75% of all points in this range were in the CEG A zone. In overt hypoglycemia (YSI values less than 3.5 mmol/dL), greater than 90% of temporally matched new CGM values were less than 4.3 mmol/l. The average coefficient of variation was 6.6%, better than previous published CGM results and within the reported precision of some BG meters. The new CGM has a high degree of reliability as measured by the percentage of sensors lasting for the full 7 days of intended use and the percentage of potential values (up to once every 5 minutes) that were actually displayed. 94% of sensors that were successfully inserted lasted the entire 7 days of wear. 98% of potential glucose values were displayed to subjects.

**Conclusion:** The new CGM demonstrates improved performance and reliability compared to currently marketed CGM devices and should provide greater confidence to patients using CGM as part of their clinical decision making. This may result in greater patient adoption and retention and further improve clinical outcomes.



Clinical Trial Registration Number: NCT01514292

## 1047

### A new and innovative method for objective evaluation of CGM profiles to structure diagnosis and therapy in type 2 diabetes

E. Salzsieder<sup>1,2</sup>, P. Heinke<sup>1</sup>, L. Vogt<sup>2</sup>, P. Augstein<sup>1,2</sup>;

<sup>1</sup>Institute of Diabetes „Gerhardt Katsch“ Karlsburg, <sup>2</sup>Diabetes Service Center, Karlsburg, Germany.

**Background and aims:** Continuous glucose monitoring (CGM) has a high potential to improve significantly diabetes care and management. However, an easy, objective and practicable tool to evaluate the quality of measured glucose profiles is missing. The aim of our study was therefore to develop an evaluation score (Q-score) that considers all aspects of CGM profiles and can be applied for a complex and objective analysis.

**Materials and methods:** 1495 registered CGM profiles provided the database for this study. First, a factor analysis addressing all quality affecting parameters (mean sensor glucose, intra- and inter daily variability, time and area above or below target range) was performed to identify factors with major impact on CGM. For each factor one parameter was selected and used for the development of the Q-score.

**Results:** This study resulted in Q-score for quality assessment of CGM profiles. To verify the Q-score two diabetes specialists (DS) diagnosed independent 729 and 194 CGM profiles of type 2 diabetic patients, respectively. The results were analysed for the inter-individual variation as well as correlated with parameters for CGM profiles such as mean sensor glucose,  $\pm$ SD, MODD. There was a high correlation between the Q-score and the results of both DS (Kendalls-Tau= 0.766 and 0.719;  $p < 0.001$ ). Both DS showed a high correlation between (Kendalls-Tau=0.763;  $p < 0.001$ ), although one DS gave a better diagnosis (McNemar-Bowker-Test  $p < 0.001$ ). To establish an easy and practicable diagnosis tool the Q-score was tested for categorisation of CGM profiles. 729 profiles were categorised by one DS in very good (Q-score:  $3.4 \pm 0.8$ ), good ( $4.9 \pm 1.2$ ), satisfactory ( $7.1 \pm 1.6$ ), borderline ( $10.0 \pm 1.9$ ) and not satisfactory ( $13.8 \pm 2.6$ ). The Q-score was also correlated with HbA1c ( $r = 0.50$ ), mean sensor glucose (0.68), range (0.88), MODD (0.63) and time above (0.86) and below (0.24) target range.

**Conclusion:** The Q-score combines all essential quality criteria for describing blood glucose profiles in only one parameter. The Q-score is independent

of subjective opinions and can be used therefore for automatic evaluation of CGM curves. The Q-score has the potential to become a practical tool in diagnosis and therapy of type 2 diabetic patients.

## 1048

### Characteristics of real-life glucose profiles monitored by continuous glucose monitoring system in people with normal glucose tolerance

W. Xu, J. Yan, Y. Zhu, G. Zhang, X. Yang, L. Zeng, J. Weng;

Department of Endocrinology, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

**Background and aims:** Normal glucose tolerance (NGT) is diagnosed based on OGTT which is considered as the “gold standard”. However, OGTT, even self-monitoring of blood glucose (SMBG) by using portable blood glucose meter, is not able to reveal the real-life glucose profiles. Therefore, detailed information of glucose profiles in people with NGT is poorly studied before continuous glucose monitoring system (CGMS) has been widely used. This study aims to investigate the characteristics of real-life glucose profiles in people with NGT by CGMS.

**Materials and methods:** 40 participants (23 males, 17 females) with NGT confirmed by OGTT were enrolled in this study. Fasting blood samples were collected for measurement of fasting plasma glucose (FPG), hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>), lipid profiles, fasting insulin, and fasting C peptide. Homoeostasis model assessment was used to estimate basal  $\beta$ -cell function (HOMA B) and insulin resistance (HOMA-IR). All participants completed a 3-day period of glucose monitoring using CGMS. The 24-h mean blood glucose (MBG), standard deviation of MBG (SDBG), mean amplitude of glycemic excursions (MAGE), largest amplitude of glycemic excursions (LAGE) and means of daily differences (MODD) were calculated with the data obtained from CGMS. The protocol and informed consent document were approved by the research ethics board of our University. All patients gave written informed consent.

**Results:** This population were  $45 \pm 11$  years old, with body mass index  $25 \pm 8$  Kg/m<sup>2</sup>, FPG  $4.9 \pm 0.3$  mmol/L, HbA<sub>1c</sub>  $5.4 \pm 0.5\%$ , triglyceride  $2.0 \pm 1.3$  mmol/L, total cholesterol  $5.1 \pm 0.9$  mmol/L, LDL-C  $3.1 \pm 0.8$  mmol/L, HDL-C  $1.3 \pm 0.3$  mmol/L. MBG, SDBG, MAGE, LAGE and MODD were  $6.0 \pm 0.7$  mmol/L,  $0.9 \pm 0.1$  mmol/L,  $1.9 \pm 0.8$  mmol/L,  $2.9 \pm 1.4$  mmol/L and  $1.1 \pm 0.1$  mmol/L, respectively. 14 NGT participants experienced asymptomatic hypoglycemia defined as glucose concentration  $< 3.9$  mmol/L by CGMS. 72.5% (29/40) participants reached glucose concentrations above 7.8 mmol/L for  $5.2 \pm 4.6$  hours. When compared with participants whose glucose concentrations within 7.8 mmol/L, in addition to presenting higher glucose concentrations (FPG:  $5.0 \pm 0.4$  vs.  $4.8 \pm 0.3$  mmol/L, MBG:  $6.4 \pm 0.7$  vs.  $5.7 \pm 0.5$  mmol/L), participants with glucose concentrations above 7.8 mmol/L showed more obvious glycemic excursions, represented by higher SDBG, MAGE, LAGE and MODD (see table). There is no difference in HbA<sub>1c</sub>, lipid profiles, fasting insulin, fasting C peptide, HOMA B and HOMA-IR between 2 groups.

**Conclusion:** CGMS provides more detailed information of real-life glucose profiles in people with NGT. 72.5% NGT participants in this study spent a considerable amount of time at glucose levels considered to be “prediabetes” or even “diabetes”, characterized by more predominant glycemic excursions.

Comparison of participants whose glucose above 7.8 mmol/L and those with glucose within 7.8 mmol/L

	FPG (mmol/L)	MBG (mmol/L)	SDBG (mmol/L)	MAGE (mmol/L)	LAGE (mmol/L)	MODD (mmol/L)
participants whose glucose concentrations above 7.8 mmol/L	$5.0 \pm 0.4$	$6.4 \pm 0.7$	$1.1 \pm 0.3$	$2.3 \pm 1.1$	$3.3 \pm 1.2$	$1.2 \pm 0.4$
participants whose glucose concentrations within 7.8 mmol/L	$4.8 \pm 0.3$	$5.7 \pm 0.5$	$0.6 \pm 0.2$	$1.1 \pm 0.3$	$2.0 \pm 1.0$	$0.9 \pm 0.3$
P Value	0.027	0.006	0.001	0.000	0.001	0.026

Supported by: National Science Fund for Distinguished Young Scholars

## 1049

## New method for measuring the velocity and magnitude of directional glucose level movement of continuous glucose monitoring

A. Konyashin<sup>1</sup>, E. Patrakeeva<sup>2</sup>;<sup>1</sup>Saint-Petersburg State Technical University, <sup>2</sup>Saint-Petersburg Medical University, Russian Federation.

**Background and aims:** At the present time, growing popularity of continuous glucose monitoring (CGM) in T1DM patients gives diabetologists great amounts of data, which can be applied to analyze patients' glucose variability and, further, estimate pathogenesis of development of diabetes complications. Moreover, glucose level movement in patients with diabetes goes in trends. Many variables influence on trends, they lead actual developments in underlying fundamental conditions. In everyday practice, diabetologists require relatively quick and easy to use method to anticipate future trends without concern for underlying causes and effects. Therefore, aim was to design method for estimating glucose variability and identifying glucose level movement trend by measuring the velocity and magnitude of directional glucose level movement of CGM.

**Materials and methods:** 8 patients with T1DM CGM was performed for consecutive 6 days. The range of glucose movement over each 4-hours interval was represented with candlestick chart (Fifth International ATTD Conference). Special LabVIEW software was developed to quantify glucose level momentum using the Relative Strength Index (RSI), one of the most popular stock market price momentum indicators. Mathematically, RSI is represented as:  $RSI = 100 - (100/(1+RS))$ , where RS is the ratio of the average of n-period glucose level increases divided by the absolute value (i.e., ignoring sign) of the average of n-period decreases. The RSI computes momentum as the ratio of higher rises to lower losses: T1DM patients which have had more or stronger glucose level rises have a higher RSI than patients which have had more or stronger falls.

**Results:** RSI is a leading indicator of a change in trend direction. In a typical cycle, glucose level begins a new uptrend with very high and rising momentum. This positive velocity gradually diminishes as glucose reaches high level, as patients use insulin for treatment. The slope of the glucose level advance lessens. Almost invariably, momentum hits its peak well before the glucose hits its ultimate high. RSI decreases more dramatically as glucose rallies begin to fall short of previous peaks on minor rally attempts, depicting a very mature phase of insulin therapy effects.

**Conclusion:** RSI can be classified as a glucose level momentum oscillator, measuring the velocity and magnitude of directional movements. Usage of RSI is a good attempt to offer an objective and orderly procedure for quantifying glucose variability and identifying glucose level movement trend. It depends solely on the changes in glucose level.



## 1050

## Simulation models for population glucose distributions and individual glucose trends

C.C. Palerm, L. Desborough;

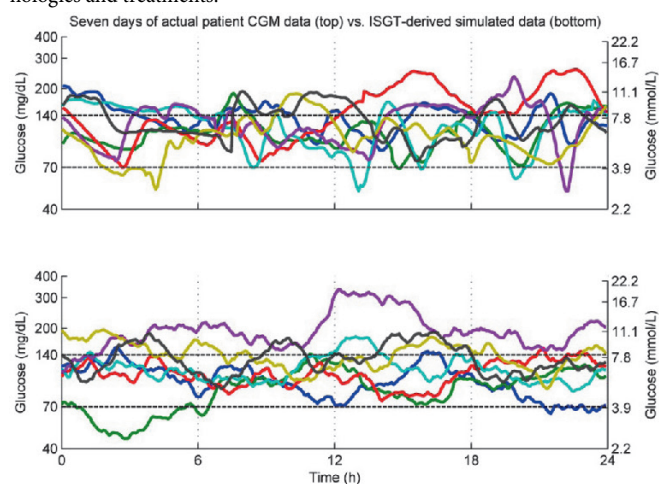
R&amp;D, Medtronic, Inc., Northridge, USA.

**Background and aims:** Development of products and services for the management of diabetes are best done using robust design methods, which requires experimentation. Models are an essential tool, as clinical trials are too time consuming and expensive for early design efforts. To accelerate development of future products and services, two simple simulation models of glycemia in people with type 1 diabetes were developed.

**Materials and methods:** The Population Static Glucose Distribution (PSGD) and Individual Subject Glucose Trend (ISGT) models were parameterized on random samples drawn from over 4000 subject-months of continuous glucose monitoring (CGM) data. Both models assume a log-normal blood glucose distribution and have been extensively validated. The PSGD was modeled using a bivariate log-normal probability distribution with zero correlation. It has four parameters: the mean ( $\mu$ ) and standard deviation ( $\sigma$ ) of both the BG values themselves and the BG standard deviation, such that  $\log_{10}(BG [mg/dL]) \sim \text{Normal}(\mu, \sigma)$  where  $\log_{10}(\mu) \sim \text{Normal}(2.09, 0.08)$  and  $\log_{10}(\sigma) \sim \text{Normal}(0.15, 0.028)$ . The notation  $X \sim \text{Normal}(\mu, \sigma)$  indicates that values of X are from a normal distribution with mean of  $\mu$  and standard deviation of  $\sigma$ . ISGT was modeled using a second order autoregressive time series driven by independent, identically distributed, white Gaussian noise. The series is first log-transformed and mean-centered. Its four parameters provide simulated CGM data. For a given  $\mu$  and  $\sigma$  from the PSGD model:  $\log_{10}(G_t) = \gamma_1 + \log_{10}(\mu) + \phi_1 y_{t-1} + \phi_2 y_{t-2} + a_t$  where  $\phi_1 = 1.6442$ ,  $\phi_2 = -0.6493$ ,  $a_t \sim \text{Normal}(0, \sigma_a)$ , and  $\sigma_a = \log_{10}(\sigma)((1 + \phi_2)/(1 - \phi_2)^2 - \phi_1^2)^{0.5}$ .

**Results:** The PSGD model has been used to estimate population glycemic variability and hypoglycemia risk, to evaluate and develop glycemia metrics, and to design "virtual clinical trials" in the artificial pancreas project. A population of "virtual subjects" is created to have the same characteristics as described by the PSGD model. These individuals are created using the ISGT model, and have been used to design user interfaces and develop artificial pancreas control and prediction algorithms. Tracings of simulated CGM data from the ISGT model are indistinguishable from patient data (see Figure).

**Conclusion:** The PSGD and ISGT models have enabled model-based development, rapid prototyping, concept engineering, and robust design. These models allow for more exploration of algorithms in a shorter time than traditional techniques, and can be used to accelerate innovation in diabetes technologies and treatments.



## 1051

## Impact of study design and analytic techniques on the reported accuracy of continuous glucose monitoring (CGM) systems

P. Simpson, T. Peyser, D. Price, A. Garcia, K. Nakamura;

Dexcom, San Diego, USA.

**Background and aims:** CGM performance is an important factor determining a patient's consistent use and clinical benefit. Accuracy, reliability and ease of use should be considered by clinicians when recommending a CGM sys-

tem. Commonly used metrics are often compared across systems; however, these comparisons are challenging due to differences in reported metrics, clinical trial design, and data analysis methods. Consistency in evaluating and reporting CGM performance is important for proper assessment of systems. As an example, CGM performance is often worst on the first and last days of use and studies that minimize data from those days may be misleading. Clinical trials with limited data in the hypo- or hyperglycemic zone or limited rates of change can distort the appearance of overall sensor performance. Real time systems for commercial use by patients typically require a prospectively determined calibration curve using SMBG meters. However, data with increased frequency of calibration, a retrospective calibration optimized for each sensor, or calibration with clinical analyzers rather than SMBG can affect apparent accuracy. Some studies report median, instead of mean differences from reference glucose, negating the impact of outlier data and impacting the accuracy perception. Studies commonly exclude data, but the amount of data excluded and the criteria for exclusion often lack transparency. Some studies report combined Clarke Error Grid A and B zones as a way of improving the perceived accuracy.

**Materials and methods:** Data from a large scale pivotal trial of Dexcom's G4 CGM system was analyzed using different methods to show the effects of frequent calibration and excluding selected data on the performance metrics of the system.

**Results:** The study enrolled 72 adult subjects (83%T1DM, 17%T2DM) at 4 US centers. Patients calibrated their CGM using a self-monitored blood glucose (SMBG) meter twice per day and were in-clinic for 12 hours on days 1, 4 and 7 to have glucose manipulated and to measure Dexcom G4 performance against YSI reference venous glucose every 15 minutes. 15% of the data were in the Hypoglycemic zone (40–80 mg/dl). The data are presented as generated in the clinical trial and also post-processed with a prospective algorithm using four calibrations per day, excluding data from the hypoglycemic zone and excluding data from the first day of patient wear.

**Conclusion:** Different study designs and methods of analysis confound the ability to compare accuracy of different CGM systems and can influence the reported results by 20% or more. There is a need for standardized study designs and metrics to allow such comparisons.

	Prospective 2 cal/day All Data	Prospective 4 cal/day	Prospective 4 cal/day Exclude Hypo	Prospective 4 cal/day Exclude Day 1 & Hypo
MARD (Mean)	13%	12%	11%	9%
MARD (Median)	10%	9%	8%	7%
Clarke A	80%	85%	86%	91%
% 20/20	82%	86%	86%	91%
N	9093	8887	7555	5023

Clinical Trial Registration Number: NCT01514292

## PS 089 Continuous glucose monitoring with pumps

1052

### Routine use of personal continuous glucose monitoring system with insulin pump in Sweden

P. Lynch<sup>1</sup>, S. Attvall<sup>2</sup>, S. Persson<sup>3</sup>, C. Barsoe<sup>4</sup>, U. Gerdtham<sup>3</sup>;

<sup>1</sup>Medtronic, Tolochenaz, Switzerland, <sup>2</sup>Diabetes Centre, Sahlgrenska University Hospital, Gothenburg, Sweden, <sup>3</sup>The Swedish Institute for Health Economics (IHE), Lund, Sweden, <sup>4</sup>Medtronic Denmark A/S, Copenhagen, Denmark.

**Background:** In 2009, the Swedish Dental and Pharmaceutical Benefits Agency (TLV) concluded that personal Continuous Glucose Monitoring (CGM) when used in combination with the Medtronic Paradigm<sup>®</sup> insulin pump should be included in the Swedish reimbursement system for specific patient indications. To support ongoing reimbursement, data was collected on the clinical and economic parameters of CGM in type 1 diabetes patients in the Swedish clinical practice.

**Methods:** The study was designed as a national multi-center, non-interventional Post-Market-Release Study. Fourteen adults and pediatric centres in Sweden participated. The data were collected both retrospectively and prospectively from medical records and patient questionnaires from May to December 2011. Data was collected regarding HbA1c-level, quality of life (QoL), frequency of hypoglycaemia, and patient preferences using Willingness to Pay (WTP). The HbA1c levels were analysed in the baseline period (0–9 months prior to CGM) and in 3 month intervals after CGM treatment was initiated, reflecting 3 month cycles of routine visits to the clinics. A mean value per patient was used if several HbA1c values were available during the baseline period or any of the 3 month follow-up periods. HbA1c values are reported in NGSP% and Mmol/mol. Inclusion criteria were Type 1 diabetes patients on pump therapy for 3+ months and either an HbA1c  $\geq$  7.9% or 2+ severe hypoglycaemic events in past 12 months or 10+ SMBG tests per day on average.

**Results:** Sixty-nine patients were included in the study. Results show a gradual decrease in HbA1c level from 8.45% in the baseline period before CGM start to 7.64% after 18–21 months of CGM treatment (a -0.81% reduction). The most rapid decrease was observed in the first year of treatment (8.45% to 7.81%, a -0.64% decrease). When using the baseline HbA1c level at the time of starting CGM (available for 13 patients), the treatment resulted in a -1.4% decrease in HbA1c after 1 year of CGM treatment (9.2% baseline to 7.8% after one year). Using an OLS model, adjusting for age and gender, a significant ( $p < 0.05$ ) reduction in HbA1c was observed of -0.62% and -1.06% after 9–12 and 18–21 months of CGM treatment, respectively. The frequency of severe hypoglycaemic events was slightly higher before the start of CGM, as reported both in medical records and by the patients (medical records: 0.10 vs. 0.02 events/month in 6 months before and after CGM start, respectively,  $p = 0.0021$ ). The number of finger sticks decreased by an average of 2.69 tests per day with CGM treatment (7.11 v 4.35,  $p < 0.0001$ ). On average 4 sensors were used per month. No significant change in QoL (measured using a VAS scale) was found from baseline to follow-up. Nevertheless, evidence of a significant WTP for CGM treatment was found; 91% preferred CGM and the average WTP for CGM was SEK 727 (EUR 82) per month when including all respondents and SEK 822 (EUR 93) when including only respondents with positive WTP.

**Conclusion:** This study indicates CGM in the real world setting significantly reduces HbA1c, number of daily finger sticks, and may reduce severe hypoglycaemic events. Although no significant change in QoL was demonstrated, patients WTP for CGM is very high. This study also confirms that initial analyses demonstrating the cost-effectiveness of CGM used to obtain reimbursement in Sweden are based on decent assumptions.

Clinical Trial Registration Number: NCT01451372

Supported by: Medtronic



## 1053

**The impact of bolus calculator and wireless communication with blood glucose meter on metabolic control in children with type 1 diabetes mellitus - randomised control trial**

A. Ramotowska, A. Szybowska, M. Lipka, M. Procnier- Czaplińska, H. Trippenbach- Dulaska;  
Medical University of Warsaw, Poland.

**Background and aims:** Bolus calculator is one of the advanced functions in modern insulin pumps models. Together with wireless communication with blood glucose meter potentially facilitates achieving the target post prandial glucose levels. In this RCT authors assessed whether use of wireless communication between compatible devices: Medtronic MiniMed insulin pump and blood glucose meter Contour Link, Bayer results in more frequently bolus calculator using and what is the impact of exerting this tool on metabolic control in type 1 diabetic patients.

**Materials and methods:** 131 children (66 girls, 65 boys) with T1DM for over one year were included into analysis. The mean age was 12.9 yrs (7-17, SD 2.8), mean diabetes duration 5 yrs (1- 14.2, SD 3.2), mean HbA1c 7.1% (5-10.2, SD 1). Subjects were randomly assigned to the one of three groups: A- patients using bolus calculator wirelessly communicated with blood glucose meter, B- patients using bolus calculator without communication with blood glucose meter or C- control group. The primary outcomes were HbA1c level and post prandial glucose level.

**Results:** HbA1c level did not significantly differ between the groups after the 3 months of observation: group A vs B (median 7.05 vs 7.2%;  $p=0.247$ ), group B vs C (median 7.2 vs 6.9%;  $p=0.066$ ). There were also no significant differences between daily dose of insulin between the groups: group A vs B (0.82 vs 0.76 u/kg/d;  $p=0.195$ , group B vs C (0.76 vs 0.82 u/kg/d;  $p=0.212$ ). No statistically differences were observed between the groups in regard to post prandial blood glucose levels: group A vs B (151.7 vs 153.8 mg/dl;  $p=0.333$ ), group B vs C (153.8 vs 145.2 mg/dl;  $p=0.185$ ). Patients in group A use bolus Wizard statistically more frequently compared to group B patients: group A vs B (4.5 vs 2.3/day;  $p=0.002$ ). Patients using wireless communication between insulin pump and blood glucose meter were more satisfied and motivated in the diabetes management (35 vs 27 subjects;  $p=0.005$ ). Incidence of self blood glucose monitoring per day was similar in all groups : group A vs B (5.2 vs 5.5 measurements/day;  $p=0.622$ ), group B vs C (5.5 vs 6.2 measurements/day;  $p=0.279$ ). There was no differences in the number of correctional boluses per day: group A vs B (34.1 vs 37.3 boluses/day;  $p=0.683$ ) as well as percentage of glucose measurements above (group A vs B 44 vs 51%;  $p=0.384$ , group B vs C 54 vs 47%;  $p=0.149$ ) and below (group A vs B 11 vs 8.8%;  $p=0.204$ , group B vs C 8.8 vs 12.2%;  $p=0.064$ ) the target.

**Conclusion:** Bolus calculator using dose not significantly influence on HbA1c level and post prandial glycaemic control but gives patients more satisfaction from the treatment. Patients using wireless communication with blood glucose meter use bolus calculator more frequently.

## 1054

**Feasibility of adjacent insulin infusion and glucose sensing via the Combo-set**

D.N. O'Neal<sup>1</sup>, S. Adhya<sup>2</sup>, A. Jenkins<sup>1</sup>, G. Ward<sup>1</sup>, J.B. Welsh<sup>2</sup>, G. Voskanyan<sup>2</sup>;  
<sup>1</sup>Department of Medicine, St Vincent's Hospital, University of Melbourne, Fitzroy, Australia, <sup>2</sup>Medtronic, Inc., Northridge, USA.

**Background and aims:** Subcutaneous insulin infusion and nearby glucose-sensing electrodes may interfere with each other. A new combination device, Combo-set, incorporates an insulin infusion catheter and a CGM sensor separated by a short distance. We evaluated insulin delivery and glucose sensing functions of this device.

**Materials and methods:** Ten adult subjects with type 1 diabetes participated in the 3-day study. Each subject had a Combo-set inserted in the abdomen, a contralateral Sof-sensor glucose sensor attached to an iPro recorder as a control, and a contralateral infusion set for routine insulin delivery. The Combo-set delivered insulin diluent except during meal tests on days 1 and 3, when boluses of insulin Lispro were delivered via the Combo-set. Post-bolus venous Lispro levels were determined at 0, 30, 60, 120, and 180 min.

**Results:** The Combo-set was well tolerated without any local skin reactions. The Combo-set sensor and control Sof-sensor had similar performance characteristics. The mean absolute relative difference (MARD) versus capillary blood glucose readings of the Combo-set sensor was similar to that of the Sof-sensor ( $p=0.63$ , NS). Clarke Error Grid analysis showed that 96.82% of

Combo-set values and 93.14% of Sof-sensor values were in the A+B regions ( $p=0.20$ , NS). Combo-set and Sof-sensor readings were comparably accurate during meal tests. Insulin via the Combo-set showed the expected post-bolus peak time ( $66.6\pm9.16$  min, mean  $\pm$  SE). The mean pre-bolus plasma insulin concentration was  $5.53\pm0.48$  mU/L and the mean post-bolus Cmax was  $52.47\pm6.09$  mU/L ( $N=17$ ). Postprandial glycemia with test meals was comparable to profiles obtained on Day 2, when subjects were on their usual diet and received insulin via the control infusion set. One "No Delivery" alarm occurred during the 21 patient-days of use, similar to the historical control rate of other infusion sets (1 per 24 patient-days in the CareLink database of 99,857 patients in 2010).

**Conclusion:** Comparable accuracy is found with glucose sensors placed near insulin infusion sites and glucose sensors placed elsewhere on the abdomen, and insulin pharmacokinetics and pharmacodynamics are unaffected by infusion catheter placement near glucose sensor insertion sites. This study shows the feasibility of simultaneous adjacent placement of an insulin infusion catheter and a CGM sensor.

## 1055

**Proposal for artificial pancreas device system-level mode logic design**

R. Kircher, R. Mauseth, D. Matheson;  
Dose Safety, Inc., Redmond, USA.

**Background and aims:** The Artificial Pancreas (AP) design continues to evolve due to advances in control algorithms, glucose sensor and insulin pump integration, and FDA guidance. This paper describes two proven strategies employed in the design the Boeing 777 Fly-By-Wire (FBW) Primary Flight Control System (PFCS) and applies those strategies to the Artificial Pancreas Device System (APDS). The main contribution of this abstract is a system specification rather than algorithmic realization.

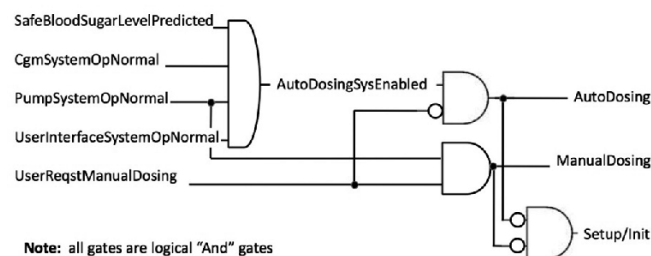
**Materials and methods:** Two design strategies of the 777 Primary Flight Control System (PFCS) are applicable to the APDS: 1) maintaining a flight control system operation that is consistent with a pilot's past training and experience, and 2) employing automated flight control mechanisms as the default while allowing the pilot to override those mechanisms on demand. This means that however different the actual flight control system architecture is from previous Boeing airplanes, the presentation to the pilot is that of a conventionally controlled mechanical system. The improved airplane handling quality and safety features are automatically provided without pilot action, yet the pilot may at any time bypass the advanced features and fly the airplane as if it had a mechanical system. The same design strategies may be applied to the APDS. For the APDS we propose design requirements that 1) preserve the user interface for the manual operation of the insulin pump and 2) enable the fully automated, closed loop control of blood sugar whenever it is safe to do so as shown on the figure. Automated dosing, the default mode, is enabled whenever: a) safe blood sugar levels are predicted; b) the CGM, pump and user interface subsystems are operating normally, and c) the user is not manually dosing. If neither manual dosing nor automated dosing is enabled, then the system goes to the Setup/Init mode. Results of simulated Use Cases and the resulting mode transition.

- 1) Predicted unsafe low blood sugar while in AutoDosing mode: Transition to ManualDosing
- 2) User infuses correction bolus while AutoDosing: Transition to ManualDosing
- 3) Predicted safe blood sugar after ManualDosing: Transition to AutoDosing
- 4) BG calibration needed while in AutoDosing mode: Remain in AutoDosing
- 5) Predicted safe blood sugar while CGM and pump operate normally in Init/Setup mode: Transition to AutoDosing
- 6) CGM sensor failure while in AutoDosing mode: Transition to ManualDosing + warning
- 7) Loss of comm. between AP device and CGM sensor while in AutoDosing: Transition to ManualDosing + warning

**Results:** The simulated execution of Use Cases utilizing the system-level APDS mode logic shows that the proposed top-level design meets the key requirements. The main contribution of this abstract is definition of high-level design requirements and a top-level APDS model logic design, based on the Boeing 777 FBW PFCS.

**Conclusion:** The 777 FBW Primary Flight Control System and the APDS have similar safety and user requirements. We believe that the proposed requirements and mode logic are suitable for APDS system development, regulatory approval, and commercialization.

### Proposed APDS Mode Logic



Note: all gates are logical "And" gates

Supported by: NIH SBIR

## 1056

### Is there a diurnal pattern to insulin action in type 1 diabetes? Implications for a closed loop system

A. Basu<sup>1</sup>, D. Nandy<sup>1</sup>, C. Dalla Man<sup>2</sup>, A. Saad<sup>1</sup>, H. Ling<sup>1</sup>, J. Levine<sup>1</sup>, S. McCrady Spitzer<sup>1</sup>, R. Basu<sup>1</sup>, C. Cobelli<sup>2</sup>, Y. Kudva<sup>1</sup>;

<sup>1</sup>Endocrinology, Metabolism and Nutrition, Mayo Clinic College of Medicine, Rochester, USA, <sup>2</sup>Information Engineering, University of Padova, Italy.

**Background and aims:** We have recently demonstrated existence of a diurnal pattern to postprandial insulin action (Si) in individuals without diabetes with greater Si at breakfast than dinner. It is critical to determine the presence/absence of such a pattern in type 1 diabetes (T1D) to inform a cutting-edge closed loop control system.

**Materials and methods:** Individuals with c-peptide negative T1D (9 men, 7 women, BMI 25.4±1.0 kg/m<sup>2</sup>, age 41.9±3.7 yrs, fasting glucose 8.9±0.8 mM, HbA<sub>1c</sub> 7.2±0.2%) on CSII with normal gastric emptying determined by scintigraphy. Identical mixed meals (10 cal/kg, 50 g carbs; 35% carbs, 30% protein, 35% fat) containing [1-<sup>13</sup>C] glucose were ingested during either breakfast (B), lunch (L) or dinner (D) at 0700, 1300 and 1900 in randomised Latin Square order on three consecutive days. Physical activity measured with triaxial accelerometry was equal on all three days. During labeled meals, [6-<sup>3</sup>H] glucose and [6,6-<sup>2</sup>H<sub>2</sub>] glucose were infused to enable concurrent measurement of rates of meal appearance (MRa), endogenous glucose production (EGP), and glucose disappearance (Rd). Insulin was administered according to individualized insulin:carbohydrate ratio for each meal.

**Results:** Postprandial glucose excursions did not differ among meals [1424.6±235 (B) vs. 899.1±246 (L) vs. 997.0±295 (D) mM/6 hours; p=0.1]. However, postprandial insulin concentration was higher (p<0.03) at B than L but did not differ among other meals. There were no detectable differences in MRa, EGP and Rd between meals. In contrast, there was a trend (p=0.1) for a lower Si at B than D (5.81±2.3 vs. 11.21±3.3 10<sup>-4</sup> dl/kg/min per μU/ml) but did not differ from L (6.85±1.22 10<sup>-4</sup> dl/kg/min per μU/ml). 11/16 subjects demonstrated lower Si at B than D.

**Conclusion:** Under controlled experimental conditions when identical meals are consumed, insulin concentrations are higher at B and a trend noted towards lower Si at B than D. However, the inter-individual variance of Si patterns precludes uniform application to all people with T1D. The results suggest that although Si varies during the day, the pattern appears to be individual specific thus suggesting that Artificial Pancreas software systems may need to be personalised.

Supported by: NIH DK 85516-01

## 1057

### Feasibility study assessing hypoglycaemia-hyperglycaemia minimiser (HHM) system in patients with type 1 diabetes in a clinical research centre (CRC)

L. Mackowiak<sup>1</sup>, D.A. Finan<sup>1</sup>, T.W. McCann<sup>1</sup>, R. Venugopalan<sup>1</sup>, H. Zisser<sup>2</sup>, H. Anhalt<sup>1</sup>;

<sup>1</sup>LifeScan, Inc., West Chester, <sup>2</sup>Sansum Diabetes Research Institute, Santa Barbara, USA.

**Background and aims:** A feasibility study was carried out in a CRC setting to assess the performance of a Hypoglycemia-Hyperglycemia Minimiser (HHM) System. This HHM System includes a continuous subcutaneous insulin infusion pump, continuous glucose monitor (CGM), and a model

predictive control algorithm with a safety module, and is run on a laptop platform.

**Materials and methods:** This study was a non-randomized, uncontrolled feasibility trial, at one CRC in the United States. The investigational system was studied for approximately 24 hours for each of 13 participants during periods of open- and closed-loop control. Insulin and food variables were adjusted to challenge and assess the system. The primary objective was to evaluate the ability of the control algorithm to predict a rise or fall in glucose above or below set thresholds, and to command the pump to increase, decrease, suspend, or resume insulin infusion accordingly. The secondary objectives were to understand the HHM System's ability to safely keep glucose levels within a target range, and to provide guidance in future development.

**Results:** The study population included 13 participants; age 24-57 years; female n = 11; BMI mean (SD) 24.7 kg/m<sup>2</sup> (5.1); duration of T1DM mean (SD) 27.2 years (13.3); HbA<sub>1c</sub> mean (SD) 7.4% (0.8). The HHM System was able to predict a rise and fall in CGM glucose, and to adjust insulin accordingly in an attempt to minimize glucose excursions. The system provided warnings in advance of significant decreases in CGM glucose. There were no safety concerns, including no diabetic ketoacidosis (DKA) or severe hypoglycemia.

**Conclusion:** In conclusion, the primary and secondary objectives of this feasibility study were met, showing that, including during periods of adjustment of food and insulin variables, the HHM System was able to respond in an attempt to keep glucose levels within a specified target range by varying insulin infusion rates based on closed-loop CGM levels. Planning for further studies is underway.

Closed-loop timeframe	Time spent < 3.8 mmol/L (70 mg/dL) by CGM
Overnight (12am - 7am)	0.1%
Post breakfast (7am - 1pm)	1.5%
Post lunch (1pm - 8pm)	0.0%
Overall	0.5%

Clinical Trial Registration Number: NCT01401751

## 1058

### Sensor-augmented pump therapy in real-life: patients reported outcomes results of the INTERPRET observational study

K. Nørgaard<sup>1</sup>, A. Scaramuzza<sup>2</sup>, N. Bratina<sup>3</sup>, N. Lalić<sup>4</sup>, P. Jarosz-Chobot<sup>5</sup>, G. Kocsis<sup>6</sup>, R. Verkauskienė<sup>7</sup>, C. De Block<sup>8</sup>, O. Carrette<sup>9</sup>, S. de Portu<sup>9</sup>, J. Castañeda<sup>10</sup>, O. Cohen<sup>11</sup>, the INTERPRET study group;

<sup>1</sup>Department of Endocrinology 541, Hvidovre University Hospital, Denmark, <sup>2</sup>Luigi Sacco Hospital, Milan, Italy, <sup>3</sup>UMC-University Children's Hospital, Ljubljana, Slovenia, <sup>4</sup>Clinic for Endocrinology CCS, Belgrade, Serbia, <sup>5</sup>Specjalistyczna Praktyka Lekarska, Gliwice, Poland, <sup>6</sup>Péterfy Hospital, Budapest, Hungary, <sup>7</sup>Kaunas University Children Hospital, Lithuania, <sup>8</sup>Antwerp University Hospital, Belgium, <sup>9</sup>Medtronic International Trading Sàrl, Tolochenaz, Switzerland, <sup>10</sup>Medtronic Bakken Research Center, Maastricht, Netherlands, <sup>11</sup>Chaim Sheba Medical Center, Tel Hashomer, Israel.

**Background and aims:** Subjective factors are important for people with diabetes because the disease is primarily self-managed and self-management regimens affect virtually all aspects of daily life. The aim of the study was to evaluate, in real life setting, the impact of Continuous Glucose Monitoring (CGM) on patients reported outcomes.

**Materials and methods:** INTERPRET is a prospective multi-centre observational study, enrolling children and adults with type 1 diabetes (T1DM) who are adding CGM to insulin pump therapy (CSII) after more than 6 months on CSII. Sensors are expected to be used at least 10% of the time during one year. Diabetes Treatment Satisfaction Questionnaire status and change versions (DTSQs and DTSQc) and Fear of Hypoglycemia (HFS) questionnaires were collected at baseline and at 12 months.

**Results:** 263 T1DM patients (mean age: 28.0 ± 15.7 years; diabetes duration: 13.9 ± 10.7 years; CSII duration: 2.6 ± 3 years and sensor usage: 30%) were eligible. After one year follow-up treatment satisfaction remained unchanged with high positive scores (32 out of 36). CGM was associated with a significant decrease in the perceived frequency of hyperglycemic episodes (p=0.047). DTSQc questionnaire, with all patients reporting positive score at 12 months follow up, is indicating an improvement in treatment satisfaction compared with previous treatment; the perceived frequency of hyperglycemia

as and hypoglycemia was also reduced with CGM. Total HFS score was significantly improved versus baseline after 12 months of treatment (37.00 vs 28.95, -6.9 adjusted decrease,  $p=0.003$ ) and both, behaviors to avoid hypoglycemia and worries about hypoglycemia, subscales were significantly improved over the 12 months of treatment with CGM (16.03 vs 13.35, -2.6 adjusted decrease,  $p=0.024$  and, 23.39 vs 18.40, -4.1 adjusted decrease,  $p=0.003$ , respectively).

**Conclusion:** This is the largest and longest international observational study providing real-life data on Sensor Augmented Pump. This study indicates that CGM led to a significant decrease in patient's fear of hypoglycemia and provides additional evidence on the positive impact of CGM on patient's treatment satisfaction.

*Supported by: Medtronic*

## 1059

### Use of predictive alerts in the Revel insulin pump

F.R. Kaufman, P. Agrawal, J.B. Welsh, B. Kannard;  
Medtronic, Inc., Northridge, USA.

**Background and aims:** The Revel sensor-augmented insulin pump allows for setting high and low predictive alerts at threshold sensor glucose values between 40 and 400 mg/dL, with predictive horizons from 5 to 30 min. We analyzed CareLink data to understand typical alert settings and how patients respond following alert activations.

**Materials and methods:** Data from 3642 Revel insulin pump users with predictive alerts activated during the month of January, 2012 were found in the CareLink database. There were a total of 72,153 patient-days analyzed.

**Results:** The mean (SD) setting for the low glucose alert was 79.83 (14.49) mg/dL (4.44 [0.80] mM) and the mean time of the predictive horizon was 19.17 (6.60) min. The mean setting for the high glucose alert was 224.09 (50.60) mg/dL (12.45 [2.81] mM) and the mean time of the predictive horizon was 19.87 (7.30) min. There were 161,486 low predictive alerts (2.24 per patient-day) and 84,016 high predictive alerts (1.16 per patient-day). The Table shows the cumulative occurrence of various actions taken 10, 30 and 60 minutes after low and high predictive alerts were activated. Low alerts occurred much more frequently than high alerts, but the predictive time horizons for low and high alerts were very similar. Approximately a third of subjects obtained a fingerstick glucose value within 60 min of an alert. Carbohydrate ingestion is likely underestimated in this analysis since most subjects may not enter this into the Bolus Wizard (BW) estimation algorithm. Very few subjects initiated temporary changes to their insulin infusion rates or suspended insulin in response to low glucose alerts. By 60 min, close to half of subjects had taken a bolus of insulin in response to high glucose alerts. Use of the predictive alert feature was associated with a significantly lower percentage of SG values <50 mg/dL whether the threshold alert setting was on (0.54% vs. 0.58%, respectively,  $p<0.001$ ) or off (1.03% vs. 1.44%, respectively,  $p<0.02$ ).

**Conclusion:** Revel pump patients who use predictive alerts experience frequent alert activations, many of which lead to behaviors designed to minimize hyperglycemia and hypoglycemia. Use of the predictive alert may reduce the time spent in hypoglycemia.

Table. Actions following low and high predictive alerts

After low alert	10 min	30 min	60 min
BG meter reading	14369 (8.90%)	32722 (20.26%)	52550 (32.54%)
BW carb entry	4620 (2.86%)	13429 (8.32%)	26230 (16.24%)
Suspend insulin	2435 (1.51%)	4605 (2.85%)	6692 (4.14%)
Temp basal rate	2496 (1.55%)	4300 (2.66%)	5911 (3.66%)
After high alert	10 min	30 min	60 min
BG meter reading	9806 (11.67%)	18131 (21.58%)	26346 (31.36%)
Bolus given	19797 (23.56%)	30711 (36.55%)	38824 (46.21%)
Temp basal rate	779 (0.93%)	1298 (1.54%)	1852 (2.20%)

Cumulative occurrence (N, %) of activities and pump interactions following 161,486 low predictive alerts (upper rows) and 84,016 high predictive alerts (lower rows).

## 1060

### Clinical evaluation of a fully-automated artificial pancreas using zone-model predictive control with health monitoring system

H.C. Zisser<sup>1</sup>, E. Dassau<sup>2</sup>, R. Harvey<sup>2</sup>, W. Bevier<sup>1</sup>, L. Jovanovic<sup>1</sup>, F.J. Doyle III<sup>2</sup>;  
<sup>1</sup>Clinical Research, Sansum Diabetes Research Institute, <sup>2</sup>Chemical Engineering, University of California Santa Barbara, Santa Barbara, USA.

We evaluated a fully automated closed-loop artificial pancreas in 12 subjects (8F:4M) with type 1 diabetes (age mean $\pm$ SD 49.4 $\pm$ 10.4, diabetes duration 32.7 $\pm$ 16.0y, A1C 7.3 $\pm$ 1.2). Subjects were studied in-clinic for 25 hours. One day prior to the session, two continuous glucose sensors (DexCom, San Diego) were inserted. CGM values were used as the input of the system. Subjects arrived at 4PM, their insulin pump was discontinued and a study CSII pump (Animas, West Chester, PA) was inserted. Plasma glucose was measured every 30 min by YSI for safety. The subjects ate 2 mixed meals (50 gm CHO dinner/40 gm CHO breakfast) and underwent a 30 min exercise session at 50% of the predicted heart rate reserve at 11:30 AM. There were no meal announcements or pre-meal boli. Subjects were discharged at 6PM on the following day. A zone-model predictive controller was used to control glucose concentration. The controller used an a priori model that was initialized with the subject's total daily insulin. The controller was designed to keep the glucose between 70-180mg/dl during the day and 80-140mg/dl overnight. A predictive hypoglycemia prevention algorithm (Health Monitoring System/HMS) designed to alarm if the glucose was predicted to cross 70 mg/dl in the next 15 min was used in conjunction with the zone controller to minimize the risk of hypoglycemia. The subject was given 16 gm CHO if the HMS alarm was triggered. Time in range (YSI 70-180 mg/dl): Entire session = 81%, 12AM to 7AM = 92%, session minus postprandial/exercise periods = 92%. Time in range 80-140 mg/dl: 12 AM to 7 AM = 70%. Time spent < 70 mg/dl for entire session by YSI = 1%. During the overnight period, there were no YSI values below 70 mg/dl. There were no safety events. The HMS sent appropriate warnings to prevent hypoglycemia via SMS & MMS. The combination of the zone-MPC controller and the HMS hypoglycemia prevention algorithm was able to safely regulate glucose, including challenges of unannounced meals and moderate of exercise.

*Clinical Trial Registration Number: NCT01472406*

*Supported by: NIH*



## PS 090 Predicting pregnancy outcomes

1061

### Obstetric and perinatal outcomes in diabetic women submitted to assisted reproduction techniques

E. Moreno, S. Costa, D. Acosta, N. García, L. Cerrillos, R. Torrejón;  
Virgen del Rocio Hospital, Sevilla, Spain.

**Objectives:** To evaluate the obstetric and neonatal outcomes in pregnancies in women PGDM or GDM submitted to ART, followed between January 1, 2004 and June 30, 2010.

**Materials and methods:** We included 120 women undergoing ART (Artificial Insemination (AI) or Embryo with Transfer (IVF or IVF-ET)) with PGDM or GDM. We performed a prospective observational study analyzing age, height and weight at the beginning of pregnancy, final weight, parity, type of diabetes, type of ART, concomitant diseases, degree of metabolic control, termination of pregnancy and children's hospital admission. In each patient group also analyzed the following variables: multiple pregnancy, pathology of the first trimester, percentage of preterm births, number of induced labor, mode of delivery, cesarean section rate and reasons for such, frequency of fetal macrosomia (>4000 grams), revenues in the Neonatal Intensive Care Unit (NICU), congenital malformations, and perinatal mortality.

**Results:** We studied 120 women with age of  $35.21 \pm 4.1$  years. 97 undergoing IVF, 14 to AI, 1 to AI with donor sperm and 8 oviducts. There were 30 women with PGDM and 90 with GDM. Of all pregnancies, 66% were singleton pregnancies, 32% dichorionic twin pregnancies biamniotic, 1% monochorionic twins, and 2% trigemellar. There was 1 case of embryonal reduction. There was 3 cases of abortion: all in women undergoing IVF-ET with PGDM. The rate of prematurity was 31%, 3% of very preterm and 28% between weeks 34 and 37. Among the cases of big preterm: 1 was to 29 weeks in a mother with DM1, who developed hypertension (HT), weight of newborn (NB) was 910 g and died on day 7 of life from sepsis; the second case was at 32 weeks for premature rupture of membranes, born a woman of 1610 gr, with favorable evolution; the third case was a very preterm in diamniotic dichorionic twin gestation that ended in cesarean section at 32 weeks and 4 days for preeclampsia and breech presentation of the first fetus. 54 (45%) pregnancies ended in caesarean section, 14 births eutocic (12%). We need to use vacuum in 10 cases (8%), 1 case with spatulas and forceps in 6 cases (5%). There were 9 twin births (8%). There were also 23 cases of extraclinical births, of which we have no information. The most frequently causes of cesarean were failure of induction by 20%, pelvic-cephalic disproportion in 15%, and twin pregnancy by 13%. Data regarding neonatal birth weight was as follows: 1% <1499 g, 6% between 1500 to 2499 g, 47% between 2500 to 3499 g, 29% between 3500 to 3999 g, 5%  $\geq 4000$  g and 12% with unknown data. 23 newborn (19%) required admission to the NICU: 6 for low birth weight, 1 for intrauterine growth retardation, 5 hypoglycemia, 5 of prematurity, 2 for distress, 2 for neonatal jaundice, 1 for suspected coarctation aortic and 1 caudal regression syndrome. About perinatal morbidity and mortality we observed 1 Down syndrome, 1 case of fracture clavicle, 1 postnatal death from sepsis in a very premature, 1 anal malformation, 1 coarctation of the aorta, 1 caudal regression syndrome and 1 facial asymmetry in a macrosoma fetus of 4100g, all cases in women with PGDM.

**Conclusion:** The obstetric and neonatal morbidity observed in women with PGDM undergoing ART is high, clearly related to the degree of metabolic control at the time of conception and the coexistence of visceral complications related to diabetes and / or overweight-obesity. Perinatal and neonatal loss date has been a frequent problem, and a better programming of pregnancy's a important area for improvement in care for this condition.

1062

### Gestational age at delivery in diabetic patients depending on the initial level of daily urinary albumin excretion, arterial hypertension and method of insulin injection

Z.R. Alimetova<sup>1</sup>, F.V. Valeeva<sup>1</sup>, T.N. Akhmetzianova<sup>1</sup>, I.R. Galimova<sup>2</sup>;

<sup>1</sup> Hospital therapy, Kazan State Medical University, <sup>2</sup> Republic Clinical Hospital, Kazan, Russian Federation.

**Background and aims:** Evaluate the delivery time in patients with diabetes mellitus type 1 (DM) depending on the initial level of daily urinary albumin excretion, arterial hypertension and method of insulin injections.

**Materials and methods:** Pregnancy outcomes were analyzed in 56 patients with DM 1 at the age of 19 to 37 years [26 (23, 29)] years, duration of DM of 1 month to 20 years [9 (2.8, 12.5) years]. The average level of HbA1c was 6.0 (5.4, 6.9)%. Depending on the initial stage DN all pregnant women were divided into 2 groups: those with baseline severe kidney disease (proteinuric stage) (15 women) and without the DN or the initial stage DN (normal and microalbuminuria) (41 women). 26 patients received insulin in the regime of multiple subcutaneous injections of insulin (MSII), 30 pregnant women - in the mode of constant subcutaneous insulin infusion (CSII).

**Results:** In severe DN 15 women early, the median period of delivery was 34.0 (32.5, 34.5). In the second group of women with the lack of severe manifestations of the DN were 28 women out of 41 (68.3%) delivered within a median time of delivery 38 (37.3, 39.0) weeks. Preterm delivery in this group - 13 women (31.7%) was associated with the growth of the severity of preeclampsia, with a gestational age at delivery in the group of patients with no severe renal disease of 35.5 (35.0, 37.0) w., which was higher than in the group with baseline severe renal disease ( $p = 0.005$ ). In the group of pregnant women with DM 1 with AH (19 women) the pregnancy was completed earlier - 34.0 (33.0, 35.0) weeks than in the group of pregnant women with DM 1 without AH (37) - 38.0 (37.0, 39.0) w. ( $p < 0.001$ ). In the group with AH 79.0% (15) were patients with initial proteinuric stage of DN, 21.0% (4 women) - with the initial lack of, or the initial phase of DN, in the case of severe DN the pregnancy was completed on 34.0 (32.5, 34.0) w., without or the initial stage of DN - 35.5 (34.8, 36.0) w. ( $p = 0.05$ ). In the group of women with DM 1 without AH in all patients (37 women) were absent of DN or NAM initial manifestations. However, even with the same daily urinary albumin excretion in the presence of AH pregnancy was completed significantly earlier ( $p = 0.004$ ). In the group of patients without AH on the CSII delivery was on the 38.5 (38.0, 39.0) w., using MSII pregnancy was completed earlier - 38.0 (37.0, 38.0) ( $p = 0.01$ ). In the group with the initial absence or in the initial stage of DN on CSII carried pregnancy to term 38-40 w. 86.4% (19 patients), premature delivery was used in 13.6% (3 women). In a similar group at MSII carried pregnancy to term 38-40 w. were 47.4% (9 patients), early delivery in 52.6% (10 women), which is significantly different from the results of the use of CSII ( $p = 0.02$ ). In case of early termination of pregnancy in the group of patients on CSII (3 patients) median time of delivery was 36.0 (36.0, 36.5) w., using MSII - 35.5 (35.0, 37.0) w. ( $p = 0.54$ ). The median date of completion of pregnancy in patients with initially severe DN was 34.0 (33.0, 34.0) w. in a group at MSII - 34.0 (30.5, 34.5) w. ( $p > 0.05$ ). In the presence of AH on CSII pregnancy is completed at the 34.0 (33.5, 36.0) weeks, at MSII - 34.0 (32.5, 34.8) w. ( $p = 0.67$ ).

**Conclusion:** Gestational age at delivery in patients with type 1 diabetes with severe baseline DN and the presence of hypertension decreased from 38 to 32-34 weeks. CSII compared with MSII can prolong pregnancy in patients with type 1 diabetes.

1063

### Does the presence of thyroid antibodies affect the course and outcome of pregnancy in type 1 diabetic women?

V. Vasileiou, V. Sarantopoulou, G. Philippou, P. Lymberi, L. Sarika, M. Alevizaki, E. Anastasiou;

1st Endocrine Section- Diabetes Center, Alexandra Hospital, Athens, Greece.

**Introduction:** In the literature there are only three papers so far, addressing the impact of thyroid antibodies (Anti-TPO) on pregnancy in Type1 Diabetic Women (DM1) and these present conflicting results. The aim of the study is to evaluate the presence of Anti-TPO in DM1 pregnant women and whether these are related with differences in thyroid function, metabolic control and pregnancy outcome.

**Methods:** In 78 DM1 women with singleton pregnancies Anti-TPO, Anti-Tg, TSH, FT4I (T4/TBC) were measured each trimester. At each visit (every 1-2 weeks) blood glucose, HbA1c, BMI, units of insulin/Kg were recorded, as were complications and pregnancy outcome.

**Results:** 27/78 women (34.6%) presented with positive Anti-TPO Abs. Clinical data of Anti-TPO positive and negative women are as shown in the table. First trimester TSH levels were statistically different between the two groups. There were no differences in the prevalence of diabetic complications, gestational hypertension-preeclampsia, abortions or preterm deliveries.

**Conclusion:** One third of DM1 pregnant women presented with positive Anti-TPO Abs. However their presence is not related with worse metabolic control or adverse pregnancy outcome. It seems that early treatment of thyroid dysfunction and stricter metabolic control plays a more important role than the presence of thyroid antibodies with regard to the pregnancy outcome.

	Anti-TPO positive(n=21)	Anti-TPO negative(n=57)	
	x±SD	x±SD	P
Age(years)	29.1±4.5	28.8±4.9	NS
Duration(years)	11.2±8.2	11.6±7.7	NS
BMI(kg/m <sup>2</sup> )	23.7±0.9	23.9±2.9	NS
TSH 1st trimester(μIU/ml)	2.4±1.6	1.3±1.0	0.005
TSH 2nd trimester(μIU/ml)	2.5±1.8	1.7±1.1	0.0058
TSH 3rd trimester(μIU/ml)	1.5±0.8	1.7±0.9	NS
FT4I 1st trimester	95.0±12.7	101.2±20.8	NS
FT4I 2nd trimester	112.6±24.5	110.3±24.1	NS
FT4I 3rd trimester	106.1±14.4	105.1±18.1	NS
HbA1c 1st trimester(%)	6.5±1.2	6.5±1.7	NS
HbA1c 2nd trimester (%)	5.3±0.9	5.4±0.9	NS
HbA1c 3rd trimester(%)	5.2±0.8	5.2±0.6	NS
Ins 1st trimester(IU/kg)	0.7±0.3	0.7±0.2	NS
Ins 2nd trimester(IU/kg)	0.9±0.5	0.8±0.3	NS
Ins 3rd trimester(IU/kg)	1.1±0.7	1.0±0.4	NS
Birth weight(gr)	3215.0±408.3	3092.3±688.0	NS

## 1064

### Pre-pregnancy and first trimester glycaemic control determines foetal and maternal outcomes in women with type 1 and type 2 diabetes

E. Mustafa, S. Khalil, B. Kirwan, L. Carmody, T. Gallcher, M. Todd, M. Durkan, S. Hoashi, F. Dunne;  
Department of Medicine, College of Medicine, Nursing and Health Sciences, Galway, Ireland.

**Background and aims:** Pre gestational diabetes during pregnancy poses a significant adverse impact on maternal and neonatal outcomes. A structured Pre Pregnancy Care (PPC) programme aiming to achieve optimal glycaemic control in the preconception period, increase use of folic acid, screen and stabilise diabetes complications and alteration of teratogenic medications can decrease perinatal morbidity and mortality and improve maternal morbidity. As part of our ATLANTIC DIP program we are undertaking a single arm prospective study to compare foetal and maternal outcomes in women who attend (Attendees) PPC to those who decline an invitation to attend (Non-attendees).

**Materials and methods:** To date we have identified 551 women of child bearing age with Type 1 and Type 2 Diabetes. They were invited to participate in the programme at one of 4 regional PPC clinics in the region. Consented patients received a multidisciplinary care package with pregnancy specific education on glycaemic targets hypoglycaemia and dietary advice. They had a review of medications and complications and commenced on folic acid. Pregnancies were followed to term and data on maternal and infant outcomes and glycaemic control were collected via DIAMOND.

**Results:** Of the 551 women identified 162 women (30%) have consented and attended (Attendees). 90 women (59%) had Type1 and 72 patients (41%) Type 2 diabetes. There have been 102 confirmed pregnancies in Attendees. 37 women declined to attend for PPC (Non Attendees) and 37 pregnancies have been confirmed in this group. The latter group were older (33.8 v 31.3 years) than Attendees (P= 0.005) but there was no difference in duration of disease between the groups. All Attendees received folic acid (100%) and their mean HbA<sub>1c</sub> was 6.6% (5.2-8.5) prior to conception, and 6.6% (4.6%-7.8%) in the first trimester with 78% achieving a HbA<sub>1c</sub> <7% at conception. By contrast the mean first trimester HbA<sub>1c</sub> was 8% (4.8-11.7) in Non Attendees with only 38% achieving a HbA<sub>1c</sub> <7% at conception and 29% receiving folic acid (P =0.005). There was no difference in HbA<sub>1c</sub> in the second or third trimesters between the two groups (P= 0.72 and 0.07 respectively). Composite foetal morbidity (hypoglycemia, jaundice, respiratory distress and polycythemia) was significantly less in Attendees compared to Non - Attendees (P=0.04). Admission to the Neonatal Unit (NNU) was significantly higher in Non-Attendees (84%) compared to Attendees (40%) (P <0.005). There were 2 cases of intrauterine fetal death < 24 weeks (IUID) one of them in a twin pregnancy in Attendees compared to 1 case of stillbirth at 37 weeks in Non-Attendees. No congenital anomalies were seen in Attendees compared to 2 cases in Non-Attendees. There was no difference in maternal morbidities (Pre-eclampsia, Pregnancy induced hypertension, Ant partum and

postpartum haemorrhage) between the groups (P= 0.35) or in Caesarean section rates (64% v 63%).

**Conclusion:** PPC achieving better preconception and early pregnancy glycaemic control and increased folic acid uptake in Attendees is translated into a decrease in fetal mortality and morbidities resulting in an increased take home baby rate. In addition the need for admissions to NNU care was significantly reduced resulting in cost benefits. The uptake rate to PPC remains low and requires further investigation through focus groups and proposed use of social networking.

Supported by: Health Research Board

## 1065

### Evaluation of 1,5-anhydroglucitol as marker of glycaemic control and birth weight in pregnancy complicated by type 1 diabetes mellitus

N. Nowak<sup>1</sup>, K. Cyganek<sup>1,2</sup>, B. Matejko<sup>1</sup>, M.T. Malecki<sup>1,2</sup>;

<sup>1</sup>Department of Metabolic Diseases, Jagiellonian University, <sup>2</sup>University Hospital, Krakow, Poland.

**Background and aims:** 1,5-anhydroglucitol (1,5-AG) is a marker of short-term glycemic control in diabetes - it reflects the period of the last 1-2 weeks. Its excretion rate depends on the renal glucose threshold, which is decreased during pregnancy. Thus, it is not clear whether it may be used in pregnant women with diabetes mellitus. We tested pregnant patients with type 1 diabetes mellitus (T1DM) to answer a) what is the relationship between 1,5-AG level and glycemic control assessed by continuous glucose monitoring (CGM); b) whether this particle is a predictor of birth weight.

**Materials and methods:** We examined 82 pregnant women with T1DM (mean age: 29.7 years; SD=4.5, mean diabetes duration: 12.7 yrs; SD=7.4). In each trimester, 1,5-AG concentration was measured with an immunoenzymatic assay. The data from CGM device were collected in 58 pregnancies for a 7-day period before blood collection for 1,5-AG and HbA<sub>1c</sub> analysis. The data were analyzed with Pearson correlation coefficients and multivariate regression. Receiver operating characteristic (ROC) curve analysis was used to evaluate third-trimester 1,5-AG level as a predictor of macrosomia (defined as birth weight of 4000g or more) and large for gestational age (LGA) birth weight (≥90th percentile).

**Results:** 1,5-AG correlated significantly with glycemic indices recorded by CGM: area under the curve at 140 mg/dl (r=-0.66, p=1x10<sup>-7</sup>), average maximal glucose (r=-0.58, p=4x10<sup>-6</sup>), glucose standard deviation (r=-0.6, p=2x10<sup>-6</sup>) and mean glucose (r=-0.54, p=3.1x10<sup>-5</sup>). In multivariate linear regression, 1,5-AG was independently associated with the newborn's weight (p<1x10<sup>-6</sup>), this remained highly significant also after the incorporation of HbA<sub>1c</sub> into the model (p<1x10<sup>-6</sup>). In multivariate logistic regression, low 1,5-AG concentration significantly predicted LGA (OR: 0.35, 95% CI:0.17-0.68, p=0.0025) and macrosomia (OR: 0.18, 95% CI:0.06-0.55, p=0.0024). The area under the 1,5-AG ROC curve in the LGA prediction was 0.81 (95% CI:0.7-0.89) at a criterion of 4.75 μg/ml. The AUC in the prediction of macrosomia was 0.84 (95% CI:0.75-0.93) at 4.27 μg/ml of serum 1,5-AG.

**Conclusion:** 1,5-AG is an accurate indicator of glycemic control as assessed by CGM in pregnant women with T1DM. In addition, its level in the 3rd trimester could be prognostic for birth weight. Thus, 1,5-AG use in glycemic control monitoring as well as neonatal macrosomia and LGA prediction should be considered in this population.

Supported by: MNiSW Grant (N N407 414436)

## 1066

### The association of metabolic parameters with BMI and insulin resistance in young children beyond maternal hyperglycaemia during pregnancy

L. Bozkurt, C.S. Göbl, B. Rami, A. Luger, E. Schober, A. Kautzky-Willer;  
Medical University of Vienna, Austria.

**Background and aims:** Early experience of elevated insulin concentration during critical periods of perinatal development might contribute to a lasting malprogramming of endocrine systems regulating body weight and metabolism. The aim was to assess associations in BMI-SDS (standard-deviation-score) and IR (insulin resistance) with regulating hormones in young children of mothers affected by different types of diabetes during pregnancy.

**Materials and methods:** In a prospective study, 76 children aged 4-9 years were included for clinical examinations comprising anthropometric assessments and a fasting venous blood sample for metabolic measurements including determination of leptin, ghrelin and GDF-15. Three groups were

formed according to the diabetic status of the mother during pregnancy, i.e. pre-existing diabetes, gestational diabetes and normal glucose tolerance (NGT).

**Results:** We found significant correlations between BMI-SDS and leptin ( $r=0.61$ ,  $p<0.001$ ), ghrelin ( $r=-0.32$ ,  $p=0.005$ ), usCRP ( $r=0.48$ ,  $p<0.001$ ) and HOMA ( $r=0.36$ ,  $p=0.002$ ) in the total population. Further, level of IR as determined by HOMA was associated with leptin ( $r=0.37$ ,  $p=0.001$ ), ghrelin ( $r=-0.30$ ,  $p=0.01$ ), GDF-15 ( $r=-0.38$ ,  $p=0.001$ ) as well as BMI-SDS ( $r=0.36$ ,  $p=0.002$ ) in univariable analysis. The association of GDF-15, BMI-SDS and leptin with IR was shown to be independent by using a multiple linear regression explaining 38% of the variance. Group based analysis revealed that observed correlations depended on maternal type of diabetes; with exception of leptin to BMI, no other association occurred in NGT-children.

**Conclusion:** Our results showed an inverse correlation of GDF-15 to IR in young children exposed to diabetes in utero. This association was independent of BMI-SDS and leptin. Further studies are at need to clarify the role of GDF-15 in the development of IR.

*Supported by: Austrian Science Fund (P14515MED) to AKW*

## 1067

### Insulin pump is effective to reduce macrosomia in pregnancies complicated by type 1 diabetes

F. Lorenzini<sup>1</sup>, B. Guyard Boileau<sup>1</sup>, V. Melki<sup>2</sup>, H. Hanaire<sup>2</sup>;

<sup>1</sup>Hopital Paule de Viguier, <sup>2</sup>Hopital Rangueil, CHU Toulouse, Toulouse, France.

**Background and aims:** Insulin pump is widely used during diabetic pregnancies, despite lack of randomized studies. We decided to report our “every-day life experience” with pump (CSII) in type 1, compared with rapids analogs multiple daily injections (MDI) treatment.

**Materials and methods:** Retrospective analysis of 98 type 1 patients, successfully managed in our tertiary care center, by a multidisciplinary team. 3 groups are described: G1 n=55 treated with pump before pregnancy (6 months to 12 years), G2 n=25, in whom pumps began during pregnancy (6 to 36 Weeks), and G3 n=18 remained on MDI.

**Results:** Diabetes is older and more complicated in G1 ( $p<0,03$ ), with more hypertension, retinopathy nephropathy, and laser treatment. HbA1c is better in G1 vs G2 and G3 at conception (6,79, 7,65 and 7,5%  $p<0,003$ ) and throughout pregnancy (second trimester: 5,91, 6,27, 6,51  $p=0,003$ ), to delivery (5,97, 6,38, 6,32  $p=0,03$ ). No differences in term (37,3WA), birthweight (3231/3201/3310g). But there is significantly less macrosomia in pump groups (16,4 vs 20 and 30%  $p<0,05$ ), suggesting best control with CSII in late pregnancy. Insulin doses tend to be smaller in G1 (1U/Kg vs 1,2 and 1,3) No keto acidosis, 3 episodes of ketosis in G2, none in other groups. Frequency of severe hypoglycemia, related to diabetes duration, is not different in 3 groups.

**Conclusion:** Insulin pump is effective in diabetic pregnancy, especially in older and severe diabetes to reduce HbA1c and macrosomia, without side effects when started before conception.

*Supported by: Novo Nordisk*

## 1068

### Glycaemic control and weight gain in post-pregnancy follow-up of type 1 diabetic women

K. Cyganek<sup>1</sup>, A. Hebda-Szydło<sup>1</sup>, J. Skupien<sup>2</sup>, I. Janas<sup>1</sup>, J. Walczyk<sup>1</sup>, S. Borys<sup>1</sup>, A. Lipowska<sup>3</sup>, M. Szopa<sup>1</sup>, M.T. Malecki<sup>4</sup>;

<sup>1</sup>Department of Metabolic Disease, University Hospital, Krakow, Poland,

<sup>2</sup>Section on Genetics and Epidemiology, Joslin Diabetes Center, Boston, USA, <sup>3</sup>Emory University School of Medicine, Atlanta, USA, <sup>4</sup>Department of Metabolic Disease, Jagiellonian University, Krakow, Poland.

**Background and aims:** Glycaemic control during pregnancy complicated by type 1 diabetes mellitus (T1DM) is essential for clinical outcomes. It is well-documented that most pregnant T1DM women achieve normoglycemia; however, intensive diabetes management is associated with weight gain. There is scarce data on glycaemic control and weight change after the delivery. We aimed to examine post-pregnancy glycaemic control and weight changes in T1DM women.

**Materials and methods:** We analysed 378 singleton pregnancies in T1DM women receiving medical care at Department of Metabolic Diseases, Krakow, Poland. We identified 274 subjects that participated in an intensive diabetes

management program and had at least two follow-up visits after the delivery with HbA<sub>1c</sub> and weight measurements.

**Results:** The study subjects mean age was 27.8 years  $\pm 5.0$ , with diabetes duration of 11.6 years  $\pm 7.4$ . The mean initial HbA<sub>1c</sub> level during pregnancy was 7.0%  $\pm 1.5$ , while pre-pregnancy weight was 64.5 kg  $\pm 1.5$ , BMI 23.8  $\pm 3.2$  kg/m<sup>2</sup>. The mean HbA<sub>1c</sub> in the 3rd trimester was 5.7%  $\pm 1.0$ . We observed a mean gestational weight gain of 14.2 kg  $\pm 1.5$ . During the first 6 months after delivery, HbA<sub>1c</sub> increased by 0.9% ( $p<0.0001$ ), while weight increased by 4.7 kg compared to the pre-pregnancy weight ( $p<0.0001$ ) and BMI increased to 25.5 ( $p<0.0001$ ) compared to the pre-gestational BMI. After another 6 months, HbA<sub>1c</sub> further deteriorated by 0.3%, surpassing the HbA<sub>1c</sub> value during the last trimester ( $p<0.0001$ ). The last HbA<sub>1c</sub> recorded (more than 12 months after delivery) was 1.5% higher than during pregnancy ( $p<0.0001$ ) and not different from the pre-gestational HbA<sub>1c</sub> (7.2% vs. 7.2%,  $p=0.9$ ). The weight and BMI did not return to the pre-pregnancy level, weight was 2.5 kg and BMI 1 kg/m<sup>2</sup> higher ( $p<0.0001$ ).

**Conclusion:** In summary, in this large clinical observation, T1DM women showed substantial post-pregnancy deterioration in glycaemic control. They were also unable to return to their pre-pregnancy weight. We suggest that T1DM women require special medical care after delivery to maintain their glycemic control and weight within the therapeutic targets.

*Supported by: Polish Ministry of Science Grant No. N N407 414436*

## 1069

### Pregnancy in type 2 diabetes - sub-optimal outcomes despite optimal glycaemic control

L.A. Owens<sup>1</sup>, G. Avalos<sup>1</sup>, L. Carmody<sup>2</sup>, B. Kirwan<sup>2</sup>, F. Dunne<sup>1</sup>;

<sup>1</sup>Department of Medicine, <sup>2</sup>Department of Nursing and Health Sciences, National University of Ireland, Galway, Ireland.

**Background and aims:** Pregnancy in women with Type 2 Diabetes is associated with higher maternal and neonatal morbidity and mortality. Given the rising incidence of this condition worldwide, optimisation of clinical care is important and has been shown to improve pregnancy outcomes. This retrospective study aims to compare pregnancy outcomes in women with type 2 diabetes (T2DM) with women who are screen negative for gestational diabetes (GDM).

**Materials and methods:** The Atlantic Diabetes in Pregnancy programme (ATLANTIC DIP) aims to provide optimal care for women with diabetes before, during and after pregnancy. We now compare the demographics and pregnancy outcomes of women with known Type 2 diabetes with those who have been shown to have normal glucose tolerance (NGT) through a universal screening programme for GDM. Testing for GDM occurred between 24–28 weeks gestation and consisted of 75g 2 hour oral glucose tolerance testing using IADPSG guidelines.

**Results:** 105 women with T2DM and 105 age-matched women with NGT were included. Mean age was 33.8 years. 18% of women with T2DM had pre-pregnancy care (PPC) and half (50%) took folic acid prior to conception. These women had a mean HbA1c of 48mmol (6.5%) at their first antenatal or booking visit. Only 9% of women with Type 2 Diabetes had an HbA1c of above 53mmol (7%) at any stage during pregnancy. 71% were treated with insulin. There were more non-Caucasian women with Type 2 Diabetes than in the NGT group (31.4% vs 3.9%,  $p<0.0001$ ). Women with Type 2 diabetes were heavier, with a mean BMI of 32.9 ( $\pm 6.4$ ) kg/m<sup>2</sup> compared to 26.9 ( $\pm 3.9$ ) kg/m in the NGT group ( $P = .$ ). 43% of women with Type 2 diabetes reported a previous miscarriage, significantly higher than women with NGT (24%,  $p=0.005$ ), and 25% had a hypertensive disorder in pregnancy compared to 10% in the NGT group ( $p=0.004$ ). 61% of women delivered by caesarean section compared to 24% in the NGT group ( $p<0.0001$ ), 40% of which were considered ‘emergent’. 52% of babies from mothers with Type 2 Diabetes required care in the neonatal intensive care unit after delivery compared to 9.6% of babies born to mothers with NGT ( $p<0.0001$ ). Overall fetal outcomes were worse in pregnancies complicated by diabetes, with an 86.5% live birth rate compared to 99% in women with NGT ( $p<0.0001$ ). There was a non-significant trend towards more congenital malformations in women with diabetes ( $p=0.16$ ). Rates of macrosomia and shoulder dystocia were not higher in the group with diabetes.

**Conclusion:** Despite good glycaemic control, pregnancy outcomes remain suboptimal in this cohort of women with Type 2 diabetes. These poorer outcomes may be related to higher obesity, poor uptake of folic acid and the additional burden of hypertensive disorders in pregnancy.



# PS 091 Consequences of gestational diabetes

1070

## Relationship of the incidence of gestational diabetes with the Mediterranean diet

**B. Karamanos**<sup>1</sup>, A. Thanopoulou<sup>1</sup>, E. Anastasiou<sup>2</sup>, M. Benrubi<sup>3</sup>, S. Assaad-Khalil<sup>4</sup>, M. Bachaoui<sup>5</sup>, C. Ben Slama<sup>6</sup>, N. El Bache<sup>7</sup>, H. El Ghomari<sup>8</sup>, N. Lalic<sup>9</sup>, A. Lapolla<sup>10</sup>, C. Saab<sup>11</sup>, C. Savona-Ventura<sup>12</sup>, J. Vassallo<sup>12</sup>, M. Marre<sup>13</sup>,  
<sup>1</sup>Diabetes Centre, 2nd Department of Internal Medicine, National University of Athens, Greece, <sup>2</sup>Alexandra Hospital, Athens, Greece, <sup>3</sup>Polikliniki, Athens, Greece, <sup>4</sup>University, Alexandria, Egypt, <sup>5</sup>University, Algiers, Algeria, <sup>6</sup>University, Tunis, Tunisia, <sup>7</sup>University, Damascus, Syrian Arab Republic, <sup>8</sup>University, Rabat, Morocco, <sup>9</sup>University, Belgrade, Serbia, <sup>10</sup>University, Rome, Italy, <sup>11</sup>University, Beirut, Lebanon, <sup>12</sup>University, Valetta, Malta, <sup>13</sup>University, Paris, France.

**Background and aims:** The relationship of the Mediterranean Diet with CVD is known but there are no reports for a possible relationship with Gestational Diabetes Mellitus (GDM). We aimed to explore the relationship of dietary habits and the Mediterranean Diet with the incidence of GDM in the Mediterranean region.

**Materials and methods:** Pregnant women (n=1083) from 10 Mediterranean countries underwent a 75 g OGTT at 24 - 32<sup>nd</sup> week of gestation which was interpreted by the American Diabetes Association (ADA) 2010 and the IADPSG 2012 criteria. Dietary habits were assessed by a 78-question dietary questionnaire, previously validated against the 3-Day Diet Diary and a Mediterranean Diet Score (SCORE) was computed, reflecting the degree of adherence to the Mediterranean Diet : a higher SCORE denoting better adherence.

**Results:** GDM incidence by ADA 2010 criteria was 9.5%, while by IADPSG 2012 29.0%. GDM +ve subjects by either criterion, compared to -ve were older, had higher BMI and more relatives suffering from diabetes. After adjustment for age, BMI, diabetes in the family, weight gain during pregnancy and energy intake, ADA 2010 GDM +ve subjects compared to -ve consumed less total (286 vs 308 g/d, p=0.029) and complex carbohydrates (155 vs 171 g/d, p=0.030), while fat contributed more to the energy intake (34.6 vs 33.0 %, p= 0.038). IADPSG 2012 GDM +ve subjects consumed also less total (298 vs 310 g/d, p<0.001) and complex carbohydrates (160 vs 174 g/d, p<0.001), while fat contributed more to the energy intake (35 vs 33%, p<0.001). Fat intake was positively correlated with fasting plasma glucose (FPG) and AUC glucose, while complex carbohydrate intake was negatively correlated with FPG and AUC glucose. These correlations remained significant if GDM +ve subjects were excluded, fat vs FPG  $r = 0.185$ ,  $p < 0.001$  and vs AUC glucose  $r = 0.074$ ,  $p = 0.027$ , complex carbohydrate vs FPG  $r = -0.188$ ,  $p < 0.001$  and vs AUC glucose  $r = -0.134$ ,  $p < 0.001$ . The adherence to Mediterranean Diet judged by the SCORE, was lower in GDM +ve subjects, diagnosed either by the ADA 2010 (SCORE 5.8 vs 6.3,  $p = 0.028$ ) or the IADPSG 2012 (SCORE 5.9 vs 6.4,  $p < 0.001$ ) criteria. A higher incidence of GDM was found in the lower tertile of SCORE distribution compared to the upper tertiles, both by the ADA 2010 (12.3 vs 8.0 %, OR=1.54,  $p = 0.030$ ) and IADPSG\_2012 (32.8 vs 24.3%, OR=1.35,  $p = 0.004$ ) criteria. Moreover, SCORE was negatively correlated with FPG and AUC glucose. This correlation remained significant if GDM +ve subjects were excluded, SCORE vs FPG  $r = -0.107$ ,  $p = 0.001$  and vs AUC glucose  $r = -0.117$ ,  $p < 0.001$ .

**Conclusion:** a) the known association of GDM incidence with high fat intake is confirmed and a further association with low total and complex carbohydrate intake is ascertained b) the correlation of SCORE, fat and carbohydrate intake with glucose levels exists also in GDM negative subjects c) a Mediterranean Diet pattern of eating may protect from the development of GDM

*Supported by: Mediterranean Group for the Study of Diabetes*

1071

## The effect of GDM with the IADPSG criteria on offspring anthropometry in a multiethnic population, Norway

**K. Mørkrid**<sup>1,2</sup>, L. Sletner<sup>3,2</sup>, S. Vangen<sup>3</sup>, B. Nakstad<sup>4</sup>, A.K. Jenum<sup>1,2</sup>, K.I. Birkeland<sup>1,2</sup>;

<sup>1</sup>Endocrinology, Oslo University Hospital, <sup>2</sup>University of Oslo, <sup>3</sup>Obstetric and Gynaecology, Oslo University Hospital, <sup>4</sup>Child and Adolescents Medicine, Akershus University Hospital, Lørenskog, Norway.

**Background and aims:** Adiposity at birth or macrosomia (birth weight >4000 g) is associated with diabetes later in life. Increased adiposity at birth, not necessarily with increased birth weight, is reported in offspring to women with gestational diabetes (GDM). GDM is a risk factor for macrosomia, but most macrosomic babies are born to non-GDM women. The aim was to assess the effect of GDM with the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria on the offspring's body fat and birth weight in a multiethnic population.

**Materials and methods:** Population-based cohort study of healthy pregnant women attending primary antenatal clinics in Eastern Oslo. In total 823 (74% of the invited) women, 59% with ethnic minority origin, were included at <20 weeks' gestation, and 759 performed the 75-g OGTT at 28 weeks' gestation. GDM was defined by slightly modified IADPSG criteria: FPG  $\geq 5.1$  mmol/l or 2-h PG  $\geq 8.5$  mmol/l (no 1-h PG). The offspring's birth weight, crown-heel-length, skin fold thickness (subscapular, suprailiac, triceps, thigh) were measured within 72 hours after birth. Totally 719 mothers with OGTT data and a singleton pregnancy had an offspring measured at birth. Ethnic origin: Western Europe (97% Scandinavia), South Asia, Middle East and Others. Simple descriptive, ANOVA, chi-square tests and linear regression analyses using SPSS 19.

**Result:** The GDM prevalence was 32% overall, higher for women from South Asia ( $p < 0.001$ ) and Middle East ( $p = 0.04$ ) compared with Western Europeans. No differences were found in the offspring's sex or gestational age between GDM and non-GDM women. Increased sum of skin folds was found in the offspring of GDM women compared with the non-GDM women (18.8 vs. 17.5 mm,  $p = 0.001$ ), which remained significant after adjustments for gestational age, parity and sex ( $\beta = 1.2$ ,  $p = 0.001$ ). GDM offspring were heavier at birth compared with the non-GDM offspring (3502 vs. 3405 g,  $p = 0.02$ ), also after adjustments as previously ( $\beta = 68.3$ ,  $p = 0.06$ ), and macrosomia was found in 20% vs. 12% respectively ( $p = 0.006$ ). Stratifying on ethnicity; the offspring's subscapular skin fold was increased for the GDM vs. the non GDM group for the South Asians and Middle Easterners ( $p < 0.05$ ), not for Western Europeans. Only for the South Asians, the offspring's birth weight was higher in the GDM vs. the non-GDM group ( $p < 0.001$ ), also after adjustments as previously ( $\beta = 165.3$ ,  $p = 0.008$ ).

**Conclusion:** Women identified with GDM with the IADPSG criteria delivered larger babies with greater adiposity compared with non-GDM women. Offspring to South Asian and Middle Eastern GDM women, compared with non-GDM, had increased subscapular skin folds, a surrogate measure of visceral fat. The effect of GDM on birth weight was more prominent among South Asians.

Offspring birth measurements, divided in ethnic origin and non-GDM vs GDM. Data: mean (SD) or stated

	Western Europe n=298 (41%)		South Asia n=181 (25%)		Middle East n=104 (15%)		Others n=136 (19%)	
	non- GDM	GDM n=75 (25%)	non- GDM	GDM n=75 (41%)	non- GDM	GDM n=37 (36%)	non- GDM	GDM n=40 (29%)
Male sex, (n (%))	125 (56)	32 (43)	46 (43)	51 (68)	24 (36)	16 (43)	53 (55)	22 (55)
Gestational age, days	281 (11)	281 (13)	276 (12)	279 (12)	279 (10)	278 (10)	279 (11)	282 (12)
Birth weight, gram	3555 (492)	3582 (642)	3105 (487)	3391 (503)*	3370 (518)	3572 (483)	3410 (552)	3496 (453)
Ponderal Index, kg/m <sup>3</sup>	28.3 (2.5)	28.5 (2.8)	26.4 (2.5)	26.9 (2.3)	28.3 (2.5)	29.1 (3.4)	27.3 (2.3)	27.9 (2.4)
Macrosomia, >4000g, (n(%))	37 (17)	23 (31)*	2 (2)	8 (11)*	5 (7)	8 (22)*	14 (15)	6 (15)
Subscapular skinfold**, mm	4.4 (1.1)	4.6 (1.0)	3.9 (0.8)	4.2 (1.2)*	4.0 (0.8)	4.7 (1.0)*	4.2 (1.0)	4.8 (1.4)*
Sum skinfolds**, mm	18.2 (4.2)	19.3 (3.5)*	16.5 (3.3)	17.6 (4.3)	17.1 (3.3)	19.2 (3.6)*	17.4 (3.7)	19.6 (4.9)*

\*  $p < 0.05$  for the difference between non-GDM and GDM within each ethnic group. \*\* total n=546

## 1072

# Neonatal birth weight in women with gestational diabetes defined by International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria

V. Resi<sup>1</sup>, E. Lacaria<sup>1</sup>, A. Ghio<sup>1</sup>, G. Nosiglia<sup>2</sup>, L. Battini<sup>2</sup>, A. Carmignani<sup>2</sup>, P. Bottone<sup>2</sup>, L. Volpe<sup>1</sup>, S. Del Prato<sup>1</sup>, A. Bertolotto<sup>1</sup>;

<sup>1</sup>Endocrinology and Metabolism, <sup>2</sup>Ob/Gyn Unit II, Azienda Ospedaliero, University of Pisa, Italy.

**Background and aims:** The International Association of Diabetes and Pregnancy Study Groups (IADPSG) has recommended new criteria for diagnosis of gestational diabetes (GDM). The aim of our study was to evaluate the impact of new diagnostic criteria on the outcome of pregnancy in women with GDM.

**Materials and methods:** Out 962 unselected pregnant women screened by IADPSG criteria, 203 were diagnosed with GDM (GDM-IADPSG) (age  $34 \pm 5$  years; family history of diabetes: 19%; primiparous: 54%; previous GDM: 3.4%; pre-pregnancy BMI  $24.4 \pm 4.6$  kg/m<sup>2</sup>; BMI  $\geq 30$  Kg/m<sup>2</sup> 9.8%). HbA1c was assessed at 36 weeks of pregnancy. Maternal and fetal data were collected at delivery and perinatal time to be compared with those of 251 women with normal glucose tolerance (NGT) (age  $33 \pm 5$  years; family history of diabetes: 16%; primiparous: 51%; pre-pregnancy BMI  $22.7 \pm 3.8$  kg/m<sup>2</sup>, BMI  $\geq 30$  Kg/m<sup>2</sup> 8.4%). A random sample of women (age  $35 \pm 5$  years; family history of diabetes: 33%, primiparous: 54%; pre-pregnancy BMI  $24.1 \pm 4.7$  kg/m<sup>2</sup>, BMI  $\geq 30$  Kg/m<sup>2</sup> 8.9%) with GDM diagnosed according to ADA criteria before October 2010 (GDM-ADA) was also identified.

**Results:** At pregnancy term all GDM women had normal and comparable HbA1c levels (GDM-IADPSG:  $5.4 \pm 0.3\%$ ; GDM-ADA  $5.2 \pm 0.5\%$ ,  $p = \text{NS}$ ) with no significant difference in the caesarean delivery rate (GDM-IADPSG 42%, GDM-ADA 42.2%) compared to NGT (33%). Similarly there was no significant difference with respect to macrosomia (GDM-IADPSG (8.3%; GDM-ADA 9.2%; NGT 7.3%). Whereas there was no difference in LGA between GDM-IADPSG (0%) and NGT (1%), incidence was greater in GDM-ADA (14.2%). No difference was apparent in the Ponderal Index among the 3 GDM groups (NGT  $2.60 \pm 0.28$ ; GDM-IADPSG  $2.57 \pm 0.24$ ; GDM-ADA  $2.59 \pm 0.25$ ).

**Conclusion:** Our analysis suggests that the use of the new IADPSG criteria does not result in any further improvement in metabolic, maternal and neonatal outcomes with the exception of a decrease of LGA as compared to the ADA criteria.

## 1073

# Second trimester postload glucose level as an important predictor of low birth weight infant: Tanaka women's clinic study

Y. Yachi<sup>1,2</sup>, Y. Tanaka<sup>2</sup>, I. Nishibata<sup>3</sup>, M. Yasuhara<sup>4</sup>, K. Kobayashi<sup>4</sup>, T. Matsuoka<sup>2</sup>, A. Sugawara<sup>1</sup>, S. Kodama<sup>1</sup>, K. Saito<sup>1</sup>, H. Sone<sup>1</sup>;

<sup>1</sup>Department of Endocrinology and Metabolism, University of Tsukuba Institute of Clinical Medicine, Mito, <sup>2</sup>Department of Obstetrics and Gynecology, Tanaka Women's Clinic, Tokyo, <sup>3</sup>Nursing department, Kawasaki City College of Nursing, <sup>4</sup>Department of Obstetrics and Gynecology, Japan Maternity Fitness Association, Tokyo, Japan.

**Background and aims:** Birth weight is known to affect not only perinatal health or subsequent growth/development but also morbidity in later life. Especially low birth weight (LBW) is now recognized as an established risk for future metabolic abnormalities, including obesity and diabetes. Although it is well known that hyperglycemia is an important risk factor for macrosomia, whether maternal glucose levels are associated with incident neonatal LBW is unknown. We therefore examined the associations between maternal glycemia with normal glucose tolerance and the risk of LBW births in Japanese mothers.

**Materials and methods:** Prospectively observed were 592 consecutive singleton pregnant women without recognized diabetes before the pregnancy who initially visited the obstetric clinic before 13 weeks gestation. At the first prenatal visit and at a mean gestational age of  $27.9 \pm 1.0$  weeks, a blood sample was collected in early morning after an 8-h overnight fast. Furthermore, a 50 g glucose challenge test (GCT) was done between 26–29 weeks gestation. LBW was determined as an infant with a birth weight of less than 2500g.

**Results:** Of the 35 women who were excluded from the study, 28 had gestational diabetes mellitus and 7 had pregnancy-induced hypertension, which might affect fetal growth. Out of 557 deliveries, 22 (4%) were LBW infants at full term. By multivariate logistic regression analysis that included maternal

age, parity, BMI at first prenatal visit, height, gestational age, and sex of the newborn, we observed a statistically significant inverse association between the 1-h postload glucose level of the GCT (1hGCT) and LBW incidence (odds ratio 0.77 per 10 mg/dl increments, 95%CI, 0.63–0.95). Compared with women in the highest 3 quartiles of 1hGCT ( $>108$  mg/dl), women in the lowest quartile ( $\leq 108$  mg/dl) had a 3.92-fold (1.60–9.57) higher risk for LBW births.

**Conclusion:** A low 1hGCT level (within normal glucose range) was highly associated with an increased risk of LBW births in Japanese mothers with normal glucose tolerance.

Table 1: Risk of LBW newborns by fasting and 1-h postload glucose in 557 singleton pregnancies.

Variables	Model 1 (95% CI)	P	Model 2 (95% CI)	P
1 <sup>st</sup> Trimester fasting plasma glucose	0.97 (0.91–1.03)	0.312	0.97 (0.92–1.03)	0.349
2 <sup>nd</sup> Trimester fasting plasma glucose	0.93 (0.85–1.01)	0.412	0.94 (0.86–1.03)	0.201
2 <sup>nd</sup> Trimester 1h postload glucose	0.97 (0.95–0.99)	0.013	0.97 (0.96–0.99)	0.013
2 <sup>nd</sup> Trimester 1h postload glucose categories				
10mg/dl increments	0.74 (0.61–0.90)	0.012	0.77 (0.63–0.95)	0.013
Q2–Q4: $>108$ mg/dl	1 (referent)		1 (referent)	
Q1: $\leq 108$ mg/dl	4.02 (1.79–9.89)	0.010	3.92 (1.60–9.57)	0.013

Data are Odds Ratio (95% CI). Model 1 is adjusted for parity, gestational age, and sex of the newborn. Model 2 is adjusted for parity, gestational age, sex of the newborn, maternal age, BMI at first prenatal visit, and maternal height.

Supported by: The Ministry of Health, Labour and Welfare, Japan

## 1074

# The impact of ethnicity on glucose homeostasis after gestational diabetes mellitus

C. Ignell<sup>1</sup>, N. Shaat<sup>2</sup>, M. Ekelund<sup>3</sup>, K. Berntorp<sup>2</sup>;

<sup>1</sup>Dept of Obst and Gyn, Helsingborg, <sup>2</sup>Dept of Endocrinology, Skåne University Hospital, Malmö, <sup>3</sup>Dept of Int Med, Helsingborg, all authors affiliated to Dept of Clinical Sciences, Malmö, Lund University, Sweden.

**Background and aims:** Ethnicity influences the prevalence of gestational diabetes (GDM) and its progression to manifest diabetes postpartum, being higher in non-European populations. This may partly be explained by differences in insulin secretion and action. Aims of the present study were to evaluate glucose homeostasis after GDM, the impact of ethnicity and other determinants of glucose tolerance postpartum.

**Material and methods:** Women in southern Sweden undergoing a 75 g oral glucose tolerance test (OGTT) during pregnancy in 2003–2005 were invited to follow-up postpartum. Diagnostic criteria were those defined by the WHO in 1999. At 1–2 years after delivery 470 women with GDM and 166 women with normal glucose tolerance (NGT) during pregnancy performed an OGTT with measurements of plasma glucose and insulin concentrations at fasting, 30 min and 120 min. Homeostasis model assessment (HOMA-IR) was used to estimate insulin resistance. Beta cell function was quantified as the ratio of the incremental insulin to glucose during the first 30 min of the OGTT (I/G30). The disposition index was used to adjust insulin secretion for the degree of insulin resistance ( $[I/G30]/\text{HOMA-IR}$ ). Women were grouped according to ethnicity based on stated country of origin in at least three of their grandparents. Indices were log transformed and differences in means were tested by ANCOVA, adjusting for age, parity and interval to follow-up (results given as geometric mean [95% confidence interval (CI)]). Frequency differences were tested by the Chi-square test. Multivariate logistic regression analysis was used to assess the association of known predictor variables (age, BMI, parity, first degree relative(s) with diabetes, non-European origin) with diabetes postpartum, adjusting for time to follow-up.

**Results:** Comparing women with previous GDM ( $n=470$ ) to controls (NGT during pregnancy and follow-up,  $n=150$ ), the former had higher HOMA-IR

(1.5 [1.4–1.7] vs. 1.3 [1.2–1.5],  $p=0.020$ ) and lower disposition index (8.4 [7.7–9.2] vs. 12.8 [10.8–15.2],  $p<0.001$ ). These differences were more pronounced in women with GDM who had diabetes postpartum (HOMA-IR 3.1 [2.2–4.4], disposition index 2.6 [1.9–3.7]) compared to controls ( $p<0.001$ ), while those who stayed normoglycaemic had similar HOMA-IR as controls but lower disposition index (9.6 [8.7–10.6],  $p<0.001$ ). Among women with GDM, estimates of beta cell function did not differ between non-European ( $n=94$ ) and European women ( $n=362$ ), whereas non-European women were more insulin resistant (HOMA-IR 2.0 [1.7–2.3] vs. 1.5 [1.3–1.6],  $p=0.002$ , after adjustment for BMI  $p=0.015$ ). Similarly, Arabic women ( $n=41$ ) had higher HOMA-IR (2.1 [1.6–2.7]) than European women ( $p=0.006$ ), but insignificant after adjustment for BMI. Non-European origin was associated with higher frequency of diabetes at follow-up (16%) than was European origin (4%,  $p<0.001$ ). Of the predictor variables tested for an association with diabetes after GDM, BMI and non-European origin showed the highest associations; odds ratio (95% CI), 1.1 (1.1–1.2),  $p<0.001$ , and 5.3 (1.9–14.9),  $p=0.002$ , respectively.

**Conclusions:** Women with a history of GDM display abnormalities in glucose homeostasis, also in the presence of NGT postpartum, including beta cell dysfunction and insulin resistance. These derangements may be influenced by ethnicity and BMI.

*Supported by: Zoëga, Gorthon, County of Skåne and Malmö University Hospital Foundations*

## 1075

### The features of blood glucose in gestational diabetes mellitus women during pregnancy and after delivery

Y. Li<sup>1</sup>, Y. Liu<sup>1</sup>, C. Han<sup>2</sup>, J. Li<sup>1</sup>, Y. Zhao<sup>3</sup>, L. Shi<sup>1</sup>

<sup>1</sup>Endocrinology, The Second Hospital of Jilin University, <sup>2</sup>Obstetrics and Gynecology, Obstetrics and Gynecology Hospital of Changchun, <sup>3</sup>Isotope, The Second Hospital of Jilin University, Changchun, China.

**Background and aims:** Gestational diabetes mellitus (GDM) affects mother and infant's health. And after delivery, the incidence of type 2 diabetes and metabolic syndrome of GDM women will significantly increase. In this study, we obtained blood glucose profiles of gestational diabetes mellitus (GDM) in the late second or early third trimester of pregnancy and after delivery within 6 months by the continuous glucose monitor system (CGMS), to evaluate the relationship between glycemic excursions and pregnancy outcomes and to find the characteristic of blood glucose in GDM women during pregnancy and after delivery.

**Materials and methods:** The study has been reviewed by the Local Ethics Committee. 33 pregnant women who were diagnosed with GDM in the late second or early third trimester of pregnancy were treated according to blood glucose level. The continuous glucose monitor system (CGMS) was worn for 72h after the blood glucose levels were stabilized to fasting glucose  $\leq 5.6$  mmol/L and to 2-h postprandial blood glucose  $\leq 6.7$  mmol/L during pregnancy and after delivery within 6 months. At the same time, OGTT, insulin and C-peptide level were tested.

**Results:** During pregnancy, blood glucose results monitored by CGMS had good correlation with finger-stick values ( $r = 0.876$ ,  $P < 0.01$ ), regardless of implantation in the arm or the waist, and the waist had the better correlation. The correlations between two groups were analyzed by the Pearson coefficient analysis. HbA1C levels and the mean amplitude of glycemic excursion (MAGE) significantly contributed to poor pregnancy outcomes. Adverse pregnancy outcomes-related factors were performed by a Logistic regression model.  $P < 0.05$  was considered statistically significant. After delivery, the occurrence of type 2 diabetes in GDM was related to blood glucose levels during pregnancy, regardless of the pre-pregnancy BMI, pregnancy weight gain and C-peptide levels. The blood glucose level of GDM women after delivery within 6 months was significantly lower than they were during pregnancy. And MAGE and MODD were significantly reduced in GDM women when they were after delivery.

**Conclusion:** CGMS can accurately reflect the blood glucose profile of GDM women, and waist was the best implantation site to wear CGMS. In GDM patients. The insulin treatment raised the glucose variability compared with diet only, although the patients had similar HbA1C levels, which contributed to poor pregnancy outcomes. We should strengthen the postpartum follow-up, especially in GDM women with poor glycemic control during pregnancy.

*Supported by: NSFC (NO30971398), the Technology Development Grant of Jilin Province*

## 1076

### Applicability of a combination of HbA<sub>1c</sub> and fasting plasma glucose in post-partum screening of women with gestational diabetes mellitus

D. Benaiges<sup>1</sup>, J. Chillaron<sup>1</sup>, E. Sagarra<sup>1</sup>, A. Paya<sup>2</sup>, E. Hernandez<sup>1</sup>, E. Corominas<sup>1</sup>, J. Puig<sup>1</sup>, M. Carrera<sup>1</sup>, M. Renard<sup>1</sup>, A. Mas<sup>1</sup>, C. Bosch<sup>1</sup>, A. Goday<sup>1</sup>, J. Flores<sup>1</sup>

<sup>1</sup>Endocrinology and Nutrition, <sup>2</sup>Gynecology and Obstetrics, Hospital Universitari del Mar, Barcelona, Spain.

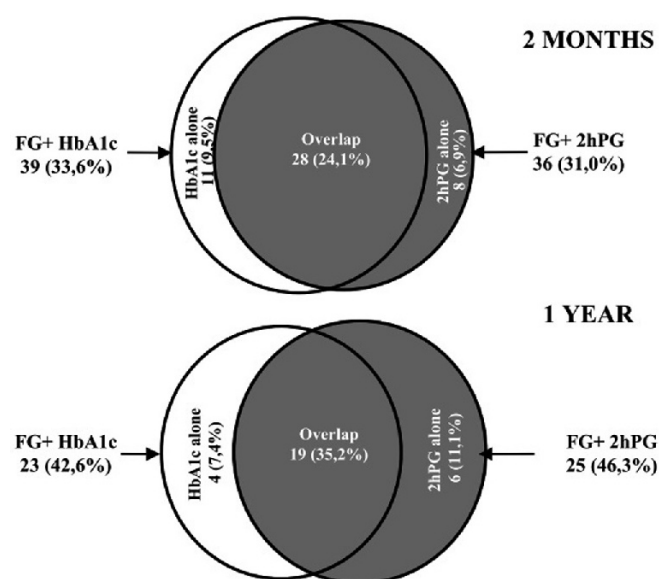
**Background and aims:** Patients with a history of gestational diabetes mellitus (GDM) have a greatly increased risk of subsequent DM. Therefore, practice guidelines recommend post-partum screening of carbohydrate metabolism and OGTT is the preferred method. A combination of HbA<sub>1c</sub>-fasting plasma glucose (FPG) has been suggested as a more practical and effective screening method. Nevertheless, little is known about the applicability of this combination for post-partum screening. The purpose of this study is to determine (a) concordance between a combination of HbA<sub>1c</sub> and FPG (HbA<sub>1c</sub>+FPG) and a combination of FPG and 2 hour plasma glucose (2hPG) (FPG+2hPG), and (b) whether substituting FPG+2hPG with HbA<sub>1c</sub>+FPG can be more cost-effective in the detection of preDM and DM in the post-partum of women with GDM.

**Materials and methods:** Prospective analysis of women diagnosed with GDM between 2006 and 2010. Women were scheduled for an OGTT and HbA<sub>1c</sub> at 2 months and 1 year post-partum. PreDM and DM were defined following the American Diabetes Association criteria. HbA<sub>1c</sub> was measured using NGSP/DCCT% units. Concordance between FPG+2hPG and HbA<sub>1c</sub>+FPG was analyzed by means of sensitivity, specificity and positive and negative predictive values. The cost of the diagnostic tests was calculated taking into account use of reagents, laboratory labour and profit and hours lost at work.

**Results:** 375 women were scheduled and 130 (34.7%) underwent post-partum screening; 116 at 2 months, 54 at one year, and 40 at both times. Women who underwent screening had a mean age of  $34.2 \pm 4.8$  years, a mean BMI of  $26.9 \pm 4.8$  Kg/m<sup>2</sup>, and 53.1% were Caucasian. No cases of DM were diagnosed at 2 months and 4 cases were diagnosed at one year. Prevalence of preDM was 40.5% at 2 months and 46.3% at one year. For overall glucose metabolism anomalies, A1c+FPG compared with FPG+2hPG had a sensitivity of 77.7%, a specificity of 86.3%, a positive predictive value of 71.8% and a negative predictive value of 89.6% at two months post-partum and 76%, 86.2%, 82.6% and 80.6% at one year, respectively. The figure shows the number of cases diagnosed with each technique (FPG+2hPG and HbA<sub>1c</sub>+FPG) at 2 months and one year post-partum. The estimated cost of using HbA<sub>1c</sub>+FPG as the screening test was 8.913 euros and the cost of OGTT was 13.386 euros (cost of hours lost at work was only included at one year screening). Use of HbA<sub>1c</sub>+FPG associates a 33% cost reduction.

**Conclusion:** HbA<sub>1c</sub>+FPG values show a good concordance with OGTT results for pre-DM and DM diagnosis, with a similar number of detected cases. Combined HbA<sub>1c</sub>+FPG has a significant cost reduction when compared with OGTT and could be a more efficient diagnostic test for post-partum screening of women with GDM. Nevertheless, the use of exclusively one of these two options underdiagnoses approximately 10% of the cases.





## 1077

### ATLANTIC DIP: the prevalence of pre-diabetes/diabetes up to 5 years post partum in women with previous gestational diabetes along the Atlantic coast

E. Noctor<sup>1</sup>, C. Crowe<sup>1</sup>, L.A. Carmody<sup>1</sup>, B. Wickham<sup>1</sup>, G. Avalos<sup>1</sup>, G. Gaffney<sup>2</sup>, P. O'Shea<sup>1</sup>, F. Dunne<sup>1</sup>;

<sup>1</sup>Department of Medicine, <sup>2</sup>Department of Obstetrics/Gynaecology, University Hospital, Galway, Ireland.

**Background and aims:** Gestational diabetes (GDM) is increasing in Ireland. Figures from our population show a 12.4% prevalence during a period of universal screening using IADPSG (International Association for Diabetes in Pregnancy Study Group) criteria. Women with GDM have a higher lifetime risk of diabetes, but most studies have involved heterogeneous populations—the applicability of this to our population is unclear.

**Materials and methods:** We invited women with a history of GDM (IADPSG criteria) in the preceding 5 years to repeat screening using a 75g OGTT to assess progression to pre-diabetes/diabetes (American Diabetes Association criteria for impaired fasting glucose/impaired glucose tolerance/ type 2 diabetes). 194 women with a GDM history were compared with 299 women with normal glucose tolerance (NGT) in pregnancy during the same period. Pearson's chi-squared test was used to estimate differences in population proportion. Logistic regression analysis was used to identify factors associated with progression to pre-diabetes/diabetes (SPSS v18).

**Results:** 14% (22/157) of GDM women rescreened had pre-diabetes/diabetes, in addition to 37 women known to have abnormal glucose tolerance within 6 months of pregnancy, giving a prevalence of 30.4% (59/194) compared to 3.3% (10/299) of women with NGT during pregnancy ( $p=0.000$ ). Index pregnancy factors associated with pre-diabetes/diabetes were; fasting glucose of 5.6 mmol/L or greater during pregnancy (OR 3.2, 95% CI 1.3,7.5,  $p=0.009$ ), week of gestation at GDM diagnosis (OR 0.9 per additional week, 95% CI 0.84, 0.99,  $p=0.033$ ) and insulin use (OR 2.9, 95% CI 1.3, 6.5,  $p=0.011$ ). Breastfeeding for greater than one month was associated with a reduction in risk (OR 0.47, 95% CI 0.23,0.97,  $p=0.40$ ). Factors at rescreening associated with pre-diabetes/diabetes were metabolic syndrome (ATPIII criteria-OR 5.1, 95% CI 2.5,10.6,  $p=0.000$ ), and higher HbA1c (OR 1.3 per 1mmol/mol increase, 95% CI 1.1,1.4,  $p=0.000$ ). Age at delivery, BMI, and time since delivery were not associated with pre-diabetes/diabetes.

**Conclusion:** The high prevalence of diabetes/pre-diabetes at this short interval (mean 2.47 years, SD 1.0) suggests a robust national rescreening program level is warranted. Further economic analysis may help to clarify what form such a program should take.

## 1078

### Hypertension and end stage renal disease in women with a past history of gestational hypertension associated with diet

F. Jebunnesa<sup>1</sup>, K. Jahan<sup>2</sup>, S. Sultana<sup>3</sup>, H. Chowdhury<sup>4</sup>, L. Ali<sup>5</sup>;

<sup>1</sup>Dept of Biochemistry & Cell Biology, Bangladesh Institute of Health Sciences, <sup>2</sup>Gynecology & Obstetrics, Shahid Showrawardy Hospital,

<sup>3</sup>Health Informatics, Bangladesh Institute of Health Sciences, <sup>4</sup>Biostatistics, Bangladesh Institute of Health Sciences, <sup>5</sup>Biochemistry and Cell Biology, Bangladesh Institute of Health Sciences, Dhaka, Bangladesh.

**Background and aims:** We have previously shown showed that Pregnancy Induced Hypertension (PIH) is associated with hypertension in a later period after delivery. The present study was undertaken to test the hypothesis that dietary factor(s) are implicated in the association between GH and later life hypertension

**Materials and methods:** Under a cross sectional design and a total of 140 women (age, 32.4±8.1 yrs, and BMI 25.1±4.1, kg/m<sup>2</sup>, M±SD) with a previous history of PIH in any pregnancy were included. Clinical, anthropometric and biochemical parameters were measured by standard techniques. Systolic blood pressure of 130 mmHg, diastolic blood pressures of 90 mmHg or mean blood pressure of >150mmHg were taken as cut-off values for hypertension (Htn). Dietary intakes were assessed by Food Frequency Questionnaire (24 hr recall methods).

**Results:** Out of 140 women 49 (35%) developed Htn and 46 (32.9%) developed ESRD over a duration of 5 to 12 yrs. Forty five (32%) of the subjects had both the complications. The hypertensive subjects (Htn) had higher uric acid (mg/dl, 7.38±1.1 vs 4.6±1.6;  $p<0.001$ ) and total protein (mg/dl, 125.3±43.6 vs 57±20.8;  $p<0.001$ ) compared to the Non-Htn subjects. Total energy intake was higher in Htn [kcal, 2175.7±399.6] as compared to non-Htn [1239±483] subjects. The higher energy was contributed mainly by carbohydrate sources from rice (g/day, 582.1±6 vs 239±71.9). On logistic regression analysis, htn showed a strong positive association with past history of PIH and total energy intake when the effects of age and BMI, past history of Htn, serum uric acid and Triglyceride were adjusted.

**Conclusion:** Women with past history of PIH have more chance of developing hypertension and it may have an association with increased energy intake from carbohydrate sources.

**Supported by:** WDF & BADAS

## PS 092 Biomarkers in gestational diabetes mellitus

1079

### Comparative evaluation of the periodontal status and plaque microbial flora in gestational diabetic and non-diabetic pregnant women: a clinico-microbiological study

S.V. Kothiwale<sup>1</sup>, V.A. Kothiwale<sup>2</sup>, A. Kumar<sup>1</sup>, P.V. Bhargava<sup>1</sup>;

<sup>1</sup>Periodontics, KLE V.K Institute of Dental Sciences, <sup>2</sup>General Medicine, Jawaharlal Nehru Medical College, Belgaum, India.

**Backgrounds and aims:** Periodontal disease is one of the most common chronic disorders of infectious origin of the supporting structures of the teeth. It is associated with an increased risk of systemic diseases such as cardiovascular diseases, diabetes mellitus and adverse pregnancy outcomes. Maternal glucose disorders during pregnancy include gestational diabetes mellitus. Diabetic retinopathy, macrosomia are common in babies born to women with pre-existing diabetes. Despite the advances in prenatal care and public awareness, adverse pregnancy outcomes still present a major public health problem worldwide. Infection is an important contributor to complications of pregnancy. Pregnant women have been found to have an increased level of periodontal infection compared with non-pregnant women of the same age. The severity of periodontal infection during pregnancy has been found to be associated with the presence of *P. gingivalis* and *P. intermedia*, leading to an increase in the adverse pregnancy outcomes. Hence, an accurate quantification of the number of individual bacterial species in dental plaque samples is needed for understanding the contribution of bacterial etiology in gestational diabetes mellitus and non diabetic pregnant women.

**Aim:** Assessment and comparison of the periodontal status and quantitative analysis of plaque microbial flora by polymerase chain reaction (PCR) in gestational diabetes mellitus patient and non diabetic pregnant women.

**Material and methods:** Initially 800 primi pregnant women were screened. Among them 60 were selected for case control study who met with inclusion and exclusion criteria. The group A included 30 gestational diabetic pregnant women and group B included 30 healthy pregnant women. Each patient was subjected for periodontal examination using gingival index, periodontal index and probing pocket depth and microbial analysis with the plaque sample. Microbiological analysis was done by PCR. The statistical analysis was carried out by student -t Test, Chi- square test and Fisher Exact test.

**Results:** The results showed statistical significant difference in gingival index (0.007), periodontal disease index (plaque index ,0.004 and periodontal pocket depth, 0.011) between gestational diabetic group and healthy pregnant group. The blood glucose parameters between the two groups was statistical significant at P value <0.001. The presence of high value of *P. intermedia* and *P. gingivalis* was positively associated in group A at P value = 0.175 and 0.007 respectively, by PCR.

**Conclusion:** The study showed an association between periodontal disease and gestational diabetes mellitus. Periodontal disease may contribute to the development of diabetes in women with the history of gestational diabetes mellitus. If the periodontal disease is confirmed as risk factor for GDM in future studies, it might provide a window of opportunity for early interventional studies. Thus it is imperative to prevent and treat periodontal disease before or during pregnancy so as to reduce maternal and infant morbidity associated with GDM.

1080

### Factors that influence fasting ketonemia and ketonuria in gestational diabetes mellitus (GDM)

E. Anastasiou<sup>1</sup>, L. Spanou<sup>1</sup>, K. Dalakleidi<sup>2</sup>, K. Zarkogianni<sup>2</sup>, A. Papadimitriou<sup>1</sup>, K. Nikita<sup>2</sup>, M. Alevizaki<sup>1</sup>, V. Vasileiou<sup>1</sup>;

<sup>1</sup>Alexandra Hospital, <sup>2</sup>Faculty of Electrical and Computer Engineering, National Technical University of Athens, Athens, Greece.

**Background:** The use of capillary blood  $\beta$ -hydroxybutyrate (3HB), a quantitative measurement, is a more precise method than urine ketones measurement (semi-quantitative detection of acetoacetate acid) for the diagnosis of diabetic ketoacidosis. Fasting ketonuria is common during normal pregnancy while there is evidence of increased frequency among pregnant women with GDM on diet. Ketone bodies cross the placenta while 3HB has been related to impaired offspring psychomotor development. Reports with

concomitant measurement of blood and urine ketones in GDM women are lacking.

**Aim:** To compare the incidence of fasting ketonemia and ketonuria in GDM women and assess their possible relation with metabolic parameters and therapeutic interventions.

**Methods:** Blood glucose, capillary blood ketones (Glucomen LX  $\beta$ -ketone, Menarini ) and urine ketones (Ketostix, Bayer) were simultaneously measured in 280 GDM pregnant women (age: 33.0 $\pm$ 0.3 years, pre-pregnancy BMI 27.05 $\pm$ 5.86kg/m<sup>2</sup>) in serial visits (total measurements: 378). 3HB levels were defined as negative (0-0.6mmol/l), intermediate (0.6-1.5mmol/l) or positive (1.5-3mmol/l). Age, prepregnancy BMI, gestational age, body weight change per gestational week, insulin treatment and bedtime carbohydrate intake were recorded.

**Results:** 3HB levels were: negative 352/378(93.1%), intermediate 20/378(5.3%) and positive 6/378(1.6%). Incidence of ketonuria was: negative 245/378 (64.8%), +/+ 84/378 (22.2%) and +++/+++ 49/378 (13%). The incidence of ketonuria was significantly higher than that of ketonemia ( $\chi^2=21.33$ ,  $p<0.001$ ). Mean 3HB levels differed significantly depending on ketonuria severity (ketonuria 0- 3HB= 0.1 $\pm$ 0.01mmol/l, (+/+) - 3HB= 0.21 $\pm$ 0.03mmol/l, (+++/+++ ) - 3HB= 0.55 $\pm$ 0.08mmol/l  $p=0.001$ ). Bed-time carbohydrate intake was associated with significantly lower 3HB levels compared to omission of the snack (3HB= 0.16 $\pm$ 0.02 vs 0.23 $\pm$ 0.03mmol/l,  $p=0.035$ ). Insulin treatment was associated with significant 3HB reduction (0.15 $\pm$ 0.02 vs 0.22 $\pm$ 0.03mmol/l,  $p=0.032$ ). Body weight reduction/week between two serial visits was associated with increased 3HB levels compared to weight gain/week (3HB 0.23 $\pm$ 0.05 vs 0.10 $\pm$ 0.02mmol/l,  $p=0.005$ ). Age correlated positively with 3HB ( $r=0.16$ ,  $p=0.002$ ). Multiple linear regression analysis showed that weight loss remained the only independent predictor of 3HB levels when age, BMI, insulin treatment and bedtime carbohydrate intake were taken into account ( $p=0.006$ ). The influence of meal intake and insulin treatment was no longer observed. Finally no correlation between 3HB and age, gestational age, parity, blood glucose levels or HbA1c was observed.

**Conclusion:** Abnormally high blood ketone levels were observed in a minor percentage (1.6%) of pregnant women with GDM, in contrast to the high presence of ketonuria (35.2%). The clinical significance of the small increase of 3HB levels, although within normal limits, in pregnant women whose body weight was reduced, requires further investigation.

1081

### The type 2 diabetes genetic risk variant TCF7L2 rs7903146 is differentially associated with gestational diabetes: differences between central and Mediterranean Europeans

L. Potasso<sup>1</sup>, N. Perakakis<sup>1</sup>, A. Lamprinou<sup>1</sup>, E. Polyzou<sup>2</sup>, D. Kassanos<sup>2</sup>, A. Peter<sup>3</sup>, R. Rasenack<sup>4</sup>, G. Paeth<sup>1</sup>, J. Seufert<sup>1</sup>, K. Laubner<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine II, University Hospital of Freiburg, Germany, <sup>2</sup>3rd Department of Obstetrics and Gynecology, University Hospital Attikon, Athens, Greece, <sup>3</sup>Department of Internal Medicine IV, University Hospital of Tuebingen, Germany, <sup>4</sup>Department of Gynecology and Obstetrics, University Hospital of Freiburg, Germany.

**Background and aims:** Single nucleotide polymorphisms (SNPs) within the transcription factor 7-like 2 (TCF7L2) gene are well known risk variants for type 2 diabetes. The best studied SNP is rs7903146, which is additionally associated with insulin secretion and BMI. Here we assess and compare the effect of this TCF7L2 risk variant on the development of gestational diabetes mellitus (GDM) in two populations of women of German and Greek origin.

**Materials and methods:** We genotyped the C/T polymorphism of the TCF7L2 gene variant rs7903146 in 96 unrelated women with a history of GDM (Greek n=50, German n=46), and in 93 women with type 2 diabetes mellitus (DM2) (Greek n= 43, German n=50) and in 155 non-diabetic pregnant women (Greek n=80, German n=75) as controls. The prevalences of non-risk CC, and risk TT alleles in the three groups were compared for Greek and German patients, both in combined and in separate country specific analysis sets.

**Results:** In the combined analysis sets of GDM vs non-diabetic pregnant women, the TT genotype of the TCF7L2 gene variant rs7903146 was significantly associated with an increased risk of GDM as compared to the non-risk CC allele [ $p=0.011$ ; Odds Ratio (OR)=3.17; 95%CI=1.318-7.665]. This risk association was particularly strong and driven by the Greek cohort [ $p=0.043$ ; OR=3.39; 95%CI=1.097-10.49], while a much weaker association was observed in the German cohort [ $p=0.13$ ; OR=3.26; 95%CI=0.72-14.7]. The CT genotype was similarly represented in both GDM and non-diabetic pregnant women, with no significant difference between German and Greek cohorts [ $p=0.83$ ]. No significant differences were detected between the GDM and

DM2 groups when comparing the presence of TT and CC genotype [OR = 1; p=1; 95%CI= 0.45–2.5]. As control, we found an association between TT genotype and DM2 when comparing both control groups [p=0.029; OR= 2.96; 95% CI=1.19–7.34].

**Conclusion:** Our data suggest significant variability of GDM risk conferred by the TCF7L2 rs7903146 TT allele even within two European populations of primary Caucasian origin. This novel observation could imply a much higher influence of the genetic background within the same ethnicity on the TCF7L2 associated risk for GDM than previously thought.

## 1082

### First trimester maternal weight predicts cord blood c-peptide levels while first trimester maternal fasting visfatin levels predict birth weight in normal pregnancy

G. Valsamakis<sup>1</sup>, D. Papatheodorou<sup>2</sup>, A. Margeli<sup>3</sup>, V. Bakoulas<sup>2</sup>, E. Kapantais<sup>4</sup>, I. Papassotiropoulos<sup>3</sup>, S. Kumar<sup>5</sup>, G. Mastorakos<sup>2</sup>;

<sup>1</sup>Endocrine Unit, Aretaieion University Hospital, Athens, Greece, <sup>2</sup>Endocrine Unit, 2nd Dept Obs&Gynae, Aretaieion University Hospital, Athens, Greece, <sup>3</sup>Biochemistry, Aghia Sofia Childrens Hospital, Athens, Greece, <sup>4</sup>Diabetes Obesity Metabolism, Metropolitan Hospital, Athens, Greece, <sup>5</sup>Diabetes Obesity Metabolism, Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism, Coventry, UK.

**Background and aims:** Fetal growth and metabolism depend on intra-uterine metabolic environment and pregnancy-associated insulin resistance. The role of first trimester maternal weight and adipose tissue markers into birth weight and cord blood c-peptide and adipocytokines in normal pregnancy was investigated.

**Materials and methods:** Seventy pregnant nonobese, nondiabetic Caucasian women were recruited randomly. Anthropometric measurements, 75 gr OGTT and fasting adipocytokines (visfatin, leptin, adiponectin, IL6) were performed at each of the 3 trimesters. At birth, birth weight and cord blood c-peptide, glucose, insulin, visfatin, leptin, adiponectin and IL6 in each of neonates were measured.

**Results:** First trimester maternal weight correlated positively and negatively with cord blood c-peptide (p=0.035, r=0.74) and cord blood visfatin (p=0.049, r=-0.67), respectively. First trimester insulin resistance (HOMAR) was negatively and positively correlated with cord blood visfatin (p=0.037, r=-0.90) and leptin (p=0.031, r=0.90), respectively. First trimester maternal weight was positive predictor of cord blood c-peptide (p=0.007). First trimester maternal visfatin levels were negative predictors of birth weight (p=0.017).

**Conclusion:** First trimester maternal weight and adipose tissue metabolism markers seem to be strongly associated with final birth weight and fetal insulin secretion suggesting the role of early-pregnancy maternal adipose tissue upon pregnancy metabolic environment as well as genetic predisposition from the mother.

*Supported by: Athens University Medical School*

## 1083

### Levels of the inflammatory marker YKL-40 in young adults exposed to intrauterine hyperglycaemia

L. Kelstrup<sup>1,2</sup>, T.F. Deigaard<sup>3</sup>, T.D. Clausen<sup>1</sup>, E.R. Mathiesen<sup>4,5</sup>, T. Hansen<sup>6,7</sup>, H. Vestergaard<sup>3,8</sup>, P. Damm<sup>1,8</sup>;

<sup>1</sup>Center for Pregnant Women with Diabetes, Department of Obstetrics, Rigshospitalet, Copenhagen, <sup>2</sup>Danish PhD School of Molecular Metabolism, Odense University Hospital, <sup>3</sup>Center of Endocrinology and Metabolism, Department of Medicine O, Copenhagen University Hospital Herlev, <sup>4</sup>Center for Pregnant Women with Diabetes, Department of Endocrinology, Rigshospitalet, Copenhagen, <sup>5</sup>Faculty of Health Sciences, University of Copenhagen, <sup>6</sup>Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health Sciences, University of Copenhagen, <sup>7</sup>Faculty of Health Sciences, University of Southern Denmark, Odense, <sup>8</sup>Faculty of Health Science, University of Copenhagen, Denmark.

**Background and aims:** YKL-40 is a glycoprotein secreted by a variety of human cell types. YKL-40 is a marker of inflammation and is associated with diabetes, insulin resistance and atherosclerosis. Fetal exposure to intrauterine hyperglycaemia because of maternal diabetes increases the risk of overweight, the metabolic syndrome and type 2 diabetes in the adult offspring. We aimed to investigate associations between exposure to intrauterine hyperglycaemia and plasma level of YKL-40 in adult offspring.

**Materials and methods:** We studied 597 offspring, aged 18–27 years, from 4 different groups based on maternal glycemic status and genetic predisposition to type 2 diabetes: 1. Offspring of women with gestational diabetes mellitus (GDM) 2. Offspring of women with risk factors for GDM but normal glucose tolerance in pregnancy 3. Offspring of women with type 1 diabetes 4. Offspring of women from the background population. The offspring were characterised by fasting plasma levels of YKL-40, and metabolic and anthropometric measurements. Exposure variables were maternal glycaemic status as well as measures of maternal glucose levels during pregnancy.

**Results:** The median plasma YKL-40 in the total cohort was 45 ng/ml (25–75 percentiles: 35–56) without significant differences between the 4 different exposure groups. Multivariate regression analysis did not show an association between maternal glucose level during pregnancy and levels of YKL-40 in the offspring. We found increasing offspring BMI at follow-up (p < 0.0001) to be a positive predictor of YKL-40. Furthermore offspring characterised by overweight (BMI >25kg/m<sup>2</sup>) or the metabolic syndrome (IDF criteria 2006) at follow-up had significantly higher levels of YKL-40 compared with the remaining offspring, (47 vs. 44 ng/ml, p = 0.02 and 49 vs. 44 ng/ml, p = 0.003 respectively).

**Conclusion:** Fetal exposure to intrauterine hyperglycaemia was not associated with levels of YKL-40 in adult offspring of women with diabetes. Adult offspring BMI were positively associated with YKL-40 levels.

*Supported by: Augustinus Fonden, Denmark*

## 1084

### Association between small heat shock proteins antibodies and beta cells dysfunction in pregnant women with abnormal glucose tolerance

K.N. Todorova - Ananieva<sup>1</sup>, E.I. Konova<sup>2</sup>, M.A. Atanasova<sup>3</sup>, R.G. Rousev<sup>3,4</sup>, K.V. Petkova<sup>5</sup>, M.G. Genova<sup>6</sup>;

<sup>1</sup>Clinic of Endocrinology, University Hospital, Plevan, <sup>2</sup>Department of Immunology, Clinical Institute of Reproductive Medicine, Plevan, <sup>3</sup>Department of Biology and Immunology, Medical Institute, Plevan, <sup>4</sup>Clinical Institute of Reproductive Medicine, Plevan, <sup>5</sup>Institute of Immunology and Biology of Reproduction, Bulgarian Academy of Science, Sofia, <sup>6</sup>Clinical laboratory and Immunology, University Hospital, Sofia, Bulgaria.

**Background and aims:** The aim of this study was to evaluate the link between serum small heat shock proteins (Hsp) antibodies and changes in  $\beta$ -cell function in pregnant women with normal and abnormal glucose tolerance.

**Materials and methods:** A 75 grams Oral Glucose Tolerance Test (OGTT) was performed on 102 Bulgarian pregnant women in the middle of gestation. According to the results of blood glucose (BG), the patients were divided in three groups: (1) Normal glucose tolerance (NGT; n1=49), (2) Impaired glucose tolerance (IGT; n2=31) and (3) Gestational diabetes mellitus (GDM; n3=22). All pregnant with GDM were negative for anti GAD65 and anti-insulin autoantibodies. The levels of BG were measured in venous plasma at 0min., 60min. and 120min. The levels of immunoreactive insulin (IRI) were measured in serum by ECLIA at 0min. and 60min. and 120min. Circulating sHSP autoantibodies was investigated in sera in all pregnant at 0 min. by using the indirect ELISA. HOMA-B, HOMA-IR and Matsuda-DeFronzo insulin sensitivity index (ISI-M) were used. ANOVA was applied for multiple comparison.

**Results:** The fasting plasma BG, the mean BG and the mean basal insulin values during the OGTT increased progressively among the 3 groups. The stimulating mean levels of IRI measured at 60 min were significantly lower in pregnant with GDM than those levels in pregnant with IGT and with NGT [n3=53.9±30.7  $\mu$ IU/ml vs. n2=65.5±34.17  $\mu$ IU/ml; (P<0.04); and resp. n3=53.9±30.7  $\mu$ IU/ml vs. n1=28.7±23.3  $\mu$ IU/ml; (P<0.001)]. Anti-sHsp antibodies were detected in 5 (16.1%) serum samples of pregnant with IGT and in 12 (54.5%) serum samples from pregnant with GDM, but not found in healthy pregnant. All pregnant with positive circulating sHsp antibodies from g2 and g3 had statistically lower levels of basal and stimulating insulin after glucose loading, compared with those women from the same both groups who were negative for anti sHsp antibodies. HOMA-B in GDM group was significantly lower than HOMA-B in NGT group (n3=99.9 vs. n1=125.4; P<0.001). HOMA-IR was progressively increased from NGT group, to IGT and GDM groups. There is no statistically significant difference in HOMA-B and HOMA-IR between pregnant of group 3 with positive and negative sHsp antibodies. Mean ISI-M was highest in the NGT group (9,18), followed by that in the IGT group (4,3) and then that in the GDM group (4, 04) (overall p<0.0001). The pregnant with GDM positive for sHsp antibodies have had significantly lower mean ISI-M compared with those levels of pregnant with



GDM negative for anti sHsp antibodies [ $2.88 \pm 0.3$  vs.  $3.83 \pm 0.5$ ; ( $P < 0.001$ )]. Multivariate logistic regression analysis have shown independent association of elevated serum anti sHsp antibodies with IGT and GDM.

**Conclusion:** Serum sHsp antibodies are detected in pregnant women with GDM and IGT. They may play a role in pathogenesis of  $\beta$ -cell dysfunction, could be as an immune phenomenon, in relation to ambient insulin resistance.

## 1085

### Circulating sclerostin levels and bone markers in gestational diabetes and physiological pregnancy

E. Ceccarelli<sup>1</sup>, E. Guarino<sup>1</sup>, L. Gennari<sup>2</sup>, D. Merlotti<sup>2</sup>, I. Spagnuolo<sup>1</sup>, M. Dalfrà<sup>3</sup>, R. Nuti<sup>3</sup>, A. Lapolla<sup>3</sup>, F. Dotta<sup>1</sup>;

<sup>1</sup>Diabetes Unit, Department of Endocrine-Metabolic Sciences, Siena,

<sup>2</sup>Department of Endocrine-Metabolic Sciences, Siena, <sup>3</sup>Department of Medical and Surgical Sciences, Padova, Italy.

**Background and aims:** Several studies have highlighted the link between changes in bone metabolism and diabetes, resulting in an increased risk of developing osteoporosis. In patients with type 2 diabetes, increased circulating levels of osteoprotegerin (OPG), an inhibitor of receptor activator of RANKL-mediated osteoclastic bone resorption, and of sclerostin (SOST) have been reported. Sclerostin is a secreted Wnt antagonist produced almost exclusively by osteocytes that binds to the Low-density lipoprotein Receptor related Proteins-5 and -6 (LRP5 e LRP6), thus inhibiting the canonical Wnt/ $\beta$ -catenin signalling pathway and thereby osteoblast activity. Aim of our study was to evaluate circulating levels sclerostin and of bone turnover markers in pregnant women with gestational diabetes subjects and with normal glucose tolerant during pregnancy.

**Materials and methods:** In our study we enrolled pregnant women with gestational diabetes (GDM,  $n=46$ ) and with normal glucose tolerant (NGT,  $n=103$ ) during pregnancy. Serum concentrations of OPG, Osteocalcin (OC), C-telopeptide of type 1 collagen (CTX), 25-OH vitamin D (evaluated by RIA) and SOST (evaluated by ELISA) have been measured in GDM and in NGT between 23rd and 29th week of pregnancy. Sclerostin was measured also in a group of non-pregnant age matched fertile women (CT).

**Results:** No difference was observed between the two groups for age and BMI before pregnancy. Fasting glucose levels in GDM, as expected, were higher than in NGT ( $88 \pm 15$  mg/dl vs  $76.8 \pm 7$ ,  $p=0.02$ ). Circulating SOST levels were similar in GDM ( $13.7 \pm 0.8$  pmol/L) and in NGT ( $13.9 \pm 8.0$  pmol/L), but were significantly lower ( $p < 0.001$ ) when compared to non-pregnant control group CT ( $40.0 \pm 20.0$  pmol/L). Osteocalcin, 25-OH vitamin D and CTX serum levels did not differ between GDM and NGT ( $0.4 \pm 0.6$  ng/ml vs  $0.4 \pm 0.4$ ;  $39 \pm 19$  ng/ml vs  $33 \pm 23$ ;  $0.4 \pm 0.21$  ng/ml vs  $0.4 \pm 0.16$ , respectively). We then divided the GDM group into two subgroups on the basis of therapy: GDM in dietary treatment ( $n = 30$ ) and GDM in insulin treatment ( $n=16$ ). In the insulin treated group, SOST levels positively correlated with BMI before pregnancy ( $r = 0.5$ ;  $p = 0.05$ ). OPG levels in insulin-treated GDM were higher than in GDM treated with diet ( $9.8 \pm 4$  vs  $7 \pm 3$  pmol/l,  $p = 0.02$ ) while OC, CTX and 25-OH vitamin D levels showed no significant difference between the two GDM subgroups.

**Conclusion:** In conclusion, we have uncovered that sclerostin levels are significantly reduced in pregnancy (both GDM and NGT), in agreement with the need to promote foetal skeletal growth; moreover OPG levels are increased in patients suffering from GDM needing insulin treatment, similar to what reported in patients with type 2 diabetes.

## 1086

### The expression of genes involved in NF- $\kappa$ B activation in peripheral blood mononuclear cells of patients with gestational diabetes

B. Telejko<sup>1</sup>, M. Kuzmicki<sup>2</sup>, A. Kretowski<sup>1</sup>, N. Wawrusiewicz-Kurylonek<sup>1</sup>, D. Lipinska<sup>1</sup>, J. Pliszka<sup>2</sup>, J. Wilk<sup>1</sup>, J. Skibinska<sup>1</sup>, A. Zielinska<sup>1</sup>, J. Szamatowicz<sup>2</sup>, M. Gorska<sup>1</sup>;

<sup>1</sup>Department of Endocrinology, Diabetology and Internal Medicine,

<sup>2</sup>Department of Gynecology, Medical University of Bialystok, Poland.

**Background and aims:** It has been shown that in patients with obesity and type 2 diabetes the changes in insulin resistance are accompanied by the changes in expression of genes involved in NF- $\kappa$ B activation in peripheral blood mononuclear cells (PBMCs). Since these effects have never been studied in patients with gestational diabetes (GDM), we measured the expression

of genes associated with the activation of NF $\kappa$ B and related to immunological and endothelial function in PBMCs obtained from women with GDM and normal glucose tolerance (NGT) during pregnancy, and assessed its relationship with various indices of insulin resistance.

**Materials and methods:** Real-time PCR was performed with TaqMan chemistry (Applied Biosystems, USA) in 35 women between 24 and 28 week of gestation. All tests were repeated four weeks later and 3 months after child birth.

**Results:** In the 2<sup>nd</sup> trimester the expression of CX3CL1 and interleukin-8 (IL-8) mRNA were significantly higher ( $p=0.02$  and  $p=0.04$ , respectively), whereas IL-17 mRNA expression was lower ( $p=0.02$ ) in the women with GDM than in the subjects with NGT. In the 3<sup>rd</sup> trimester down-regulation of TNFRSF1A ( $p=0.04$ ), PPAR- $\gamma$  ( $p=0.03$ ), STAT3 ( $p=0.02$ ) and CX3CL1 ( $p=0.04$ ) mRNA expression was found in the GDM patients in comparison with the healthy pregnant women. Three months after child birth the expression of IKBK $\beta$  ( $p=0.01$ ) and SLC27A2 ( $p=0.03$ ) mRNA was up-regulated in women with the history of GDM. In patients with NGT both in the 2<sup>nd</sup> and 3<sup>rd</sup> trimester a significant increase in CX3CL1 and STAT1 mRNA expression was observed during 4 weeks ( $p=0.03$ ), whereas in the patients who developed GDM in the 3<sup>rd</sup> trimester, there was a significant decrease in CCL5 ( $p=0.03$ ), IKBK $\beta$  ( $p=0.03$ ), DPP4 ( $p=0.01$ ) and IL-17 ( $p=0.03$ ) mRNA expression in PBMCs. In the group with NGT, IL-10 mRNA expression correlated positively with the insulin secretion-sensitivity index-2 (ISSI-2) ( $R=0.57$ ,  $p=0.03$ ) and negatively with HOMA-IR ( $R=-0.62$ ,  $p=0.02$ ). In the GDM patients STAT3 mRNA expression correlated inversely with the insulinogenic index (IGI) and InsAUC30/GluAUC30 ( $R=-0.61$ ,  $p=0.003$  and  $R=-0.45$ ,  $p=0.03$ , respectively).

**Conclusion:** The changes in the expression of genes encoding immune mediators that are involved in NF $\kappa$ B inflammatory signaling pathways in the PBMCs suggest that immune cells play an essential role in mediating insulin resistance in patients with GDM.

*Supported by: Grant No. N N407 141937 from the State Committee for Scientific Research*

## PS 093 Diagnosis of gestational diabetes mellitus

1087

**How many women with gestational diabetes mellitus are missed if selective screening strategies are used?**

G.E. Avalos, L. Owens, F. Dunne;

School of Medicine, National University of Ireland Galway, Ireland.

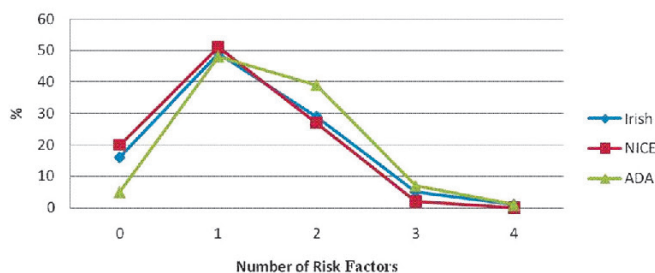
**Background and aims:** The number of women with Gestational Diabetes Mellitus (GDM) is increasing in parallel with the increase in the prevalence of obesity worldwide. There is controversy over whether universal or selective screening strategies should be undertaken. The aim of this retrospective study was to establish the number of women proven to have GDM on a universal screening programme that would otherwise have been missed using selective 'risk-factor' based screening and applying either of the American (ADA) European (NICE) or Irish guidelines.

**Materials and methods:** The ATLANTIC Diabetes in Pregnancy (DIP) programme offered universal screening for GDM to pregnant women in Ireland whose last menstrual period was between September 2006 and March 2009. 5,500 women consented and underwent a 75g Oral Glucose Tolerance Test at 24–28 weeks of gestation. The diagnosis of GDM was defined using the WHO criteria. Subsequently the dataset was re-analysed according to the International Association of Diabetes in Pregnancy Study Group [IADPSG] criteria. This dataset was used in the present study and selective screening risk factors were applied using the American Diabetes Association (ADA), National Institute for Health and Clinical Excellence (NICE) and Irish guidelines. The number of risk factors were computed for each participant using the three different guidelines. ADA guidelines: Family History of Diabetes, BMI $\geq$ 25, BP $\geq$ 140/90, and High risk ethnicity. NICE guidelines: Family History of Diabetes, BMI $>$ 30, and High risk ethnicity. Irish guidelines: Family History of Diabetes, BMI $\geq$ 30, Age $\geq$ 40 and High risk ethnicity. The three guidelines provide a list of risk factors however not all the risk factors were included in this study due to lack of information.

**Results:** Out of the 5,500 women who consented to take part in the Atlantic DIP universal screening study, 681 (12%) were diagnosed with GDM according to IADPSG criteria. 101 (16%) women diagnosed with GDM by universal screening had zero risk factors. If a selective screening strategy using Irish guidelines was used these women would have been missed. If a selective screening using the NICE guidelines was used 120 (20%) of women would be missed. Only 33 (5%) women would be missed if using the ADA guidelines.

**Conclusion:** Selective screening based on risk factors misses cases of GDM. Selective screening using 2012 ADA criteria misses fewer women (5%) with GDM. Our results agree with the recommendations of the ADA clinical practice guidelines 2012 that "All women not known to have prior diabetes should undergo a 75g OGTT at 24 to 28 weeks of gestation". If universal screening is not economically possible we should select women based on ADA 2012 selective criteria which includes women with a BMI $\geq$ 25.

**Percentage of risk factors carried by women with GDM  
n=681**



Supported by: HRB

1088

**Implications of the new criteria for diagnosis of gestational diabetes mellitus in Portugal**

J.M. Dores, C. Freitas, Pregnancy and Diabetes Study Group from the Portuguese Society of Diabetology; Endocrinology, Hospital Geral de Santo António, Porto, Portugal.

**Background and aims:** Following the recent recommendations of the International Association of Diabetes and Pregnancy Study Groups, the new criteria for diagnosis of Gestational Diabetes Mellitus (GDM) were adopted in January of 2011 in Portugal. The diagnosis can be established in either the first trimester with a fasting glucose value  $\geq$  92 mg/dl, or between the 24–28 weeks of gestation, using a 75 gr OGTT, in which is necessary to have one or more glucose values  $\geq$  92, 180 or 153 mg/dl at 0', 60' 120', respectively, to fulfill the GDM diagnosis criteria. The authors aimed to evaluate the implications of this change on the burden of their outpatient clinic, as well as the maternal-fetal outcomes.

**Materials and methods:** The authors intended to conduct a retrospective study enrolling all the Portuguese centers with GDM outpatient clinic. The authors gathered the relevant data sent by the centers after a previous invitation to participate in this observational study. The inclusion criteria was all GDM diagnosed women who were admitted in the outpatient clinic between 1<sup>st</sup> April and 30<sup>th</sup> September 2010 (group A) and in the homologous period of 2011 (group B). Both groups were compared using appropriated statistical analyses.

**Results:** Among 39 centers, 22 (56,4%) sent their data. Overall, data from 863 women were eligible for the group A and 1010 for the group B, revealing an increment of 147 cases (17%) from the first to the second group where the new GDM diagnostic criteria was applied. Comparing the 2 groups, there was a significant precocity in the time of diagnosis and referral in the group B (diagnosis at 22,5 vs 28,9 weeks;  $p=0,0001$  and first appointment at 26,3 vs 30,8 weeks;  $p<0,0001$ ). In this group pregnant women achieved less weight gain (10 vs 10,9 Kg;  $p=0,002$ ), were more insulin treated (39,8 vs 28,4%;  $p<0,0001$ ), earlier (27,9 vs 30,6 weeks;  $p<0,0001$ ) and with higher insulin dose (21,8 vs 17,9 UI/day;  $p=0,003$ ). These pregnant women had more hypertension (11,4 vs 7,2%;  $p=0,03$ ) but no preeclampsia, hidramnios or fetal death. They had more preterm delivery (9,5 vs 4,9%;  $p=0,0003$ ), but there were no differences in the type of labor between the two groups, including cesarean delivery (40% in group A vs 36,8% in Group B). Group B newborns had less mean birth weight (3135 vs 3209 gr;  $p=0,003$ ) with less large for gestational age (LGA) (12 vs 15,3 %;  $p=0,04$ ), but no statistical significant difference in macrosomia (4,4 vs 5,2%). There were no differences in the morbid-mortality of the offspring between both groups including the hypoglycemia rate (1,2% in Group A vs 1,6% in Group B). There were less reclassification tests in the group B (53,2 vs 64%) because some of the pregnancies are still in course, with no differences in their results. Nevertheless, in the group B, the results were worst in women diagnosed along the first trimester: less normal reclassification OGTT (76,9 vs 87,9%;  $p=0,003$ ), more impaired fasting glucose (5,2 vs 0,8%;  $p=0,004$ ) and more diabetes (6,7 vs 1%;  $p=0,001$ ).

**Conclusion:** The new criteria for diagnosis of GDM caused a further increment in its incidence and an earlier referral of these pregnant women to the pregnancy and diabetic clinics, so we can expect an increase in their first and second appointments. Pregnant women with GDM diagnosed in the first trimester seem to have a worst metabolic scenario according to the post partum OGTT results. Nevertheless, with an earlier and more aggressive treatment, there seems to have no differences in the outcome of the offspring.

1089

**Potential impact on service provision using different diagnostic criteria for GDM-A retrospective analysis of OGTT data in a London teaching hospital**

S. Sivappriyan<sup>1</sup>, S. Das<sup>2</sup>, D. Patel<sup>1</sup>, J. Modder<sup>2</sup>;

<sup>1</sup>Diabetes, <sup>2</sup>Obstetrics, University College London Hospitals NHS Trust, UK.

**Background:** Gestational Diabetes Mellitus (GDM) is associated with maternal morbidity, adverse perinatal and neonatal outcome and increased risk of type 2 diabetes following the index pregnancy. There is an increasing prevalence of GDM with the obesity 'epidemic'. Although diagnostic criteria for GDM was first established 40 years ago, there is still no consensus on diagnostic threshold values or number of abnormal results required. Internationally there are at least 6 different diagnostic criteria in use. Diagnosing GDM inevitably results in 'medicalisation' of pregnancy and also has resource im-

plications for healthcare commissioners and service providers. UCLH NHS Foundation Trust is a large teaching hospital in London caring for pregnant women with diabetes from varied ethnic and social backgrounds. Since 2003, we have used a three step diagnostic OGTT based on ADA criteria, following universal screening with random plasma glucose at booking and 28 weeks gestation. The International Association of Diabetes and Pregnancy Study Groups (IADPSG) recently published recommendations based on current evidence including the HAPO study. The UK National Institute of Health and Clinical Excellence (NICE) recommends criteria based on WHO recommendations.

**Aims:** Identify the number and proportion of pregnancies diagnosed as GDM using IADPSG, ADA and WHO criteria. Consider the potential resource implications for the diabetes maternity service using IADPSG criteria.

**Materials and methods:** We conducted a retrospective analysis of OGTT data for all women delivering between 1 January and 31 December 2011. We applied three most popular diagnostic criteria for GDM including WHO, ADA and new IADPSG criteria. In case of women who had more than one OGTT antenatally, only last OGTT was included for analysis.

**Results:** There were 5736 deliveries at or after 24+0 weeks between 1 Jan - 31 Dec 2011. A total of 1234 (22%) women had at least one OGTT antenatally. 414 (33 %) women had GDM using any diagnostic criteria. 153 women were diagnosed to have GDM using ADA criteria, 230 women had GDM by WHO criteria and 374 women had GDM by IADPSG criteria. Of these 124 pregnancies had GDM with all three criteria. The prevalence of GDM by ADA, WHO and IADPSG criteria was 2.7%, 4% and 6.5% respectively. In our hospital in 2011, by using ADA criteria we missed 40 GDM pregnancies diagnosed by WHO criteria, 155 GDM pregnancies diagnosed by IADPSG criteria and 66 GDM pregnancies diagnosed by both WHO and IADPSG criteria. While the prevalence of GDM women in 2011 using IADPSG criteria was much less than that in the overall HAPO study (17.8%), 221 additional GDM diagnoses would have been made if we had adopted IADPSG criteria, which represents a 144% increase in the number of GDM cases. This would have had a significant impact on maternity service provision and also long term post pregnancy follow up.

**Conclusion:** Adopting IADPSG criteria will lead to a significant increase in GDM populations. This increased prevalence appears higher than that published by another unit in UK. While IADPSG criteria is based on robust observational data, there is currently a lack of evidence about the effect of intervention on clinical outcomes in GDM diagnosed using IADPSG criteria. We are planning a retrospective comparison of clinical outcomes in the intervened versus non-intervened IADPSG pregnancies.

## 1090

### Gestational diabetes audit

A.I. Palalau<sup>1</sup>, J. Murphy<sup>2</sup>, L. Williams<sup>1</sup>, G. Chana<sup>1</sup>, N. Johns<sup>2</sup>, J. Webber<sup>1</sup>;

<sup>1</sup>Diabetes Centre, Queen Elizabeth Hospital Birmingham, <sup>2</sup>Diabetes Centre, Birmingham Women's Hospital, UK.

**Background and aims:** Gestational Diabetes (GDM) is increasingly being recognised and more at risk patients are being screened; this is likely to lead to an increase in the number of diagnoses. We aimed to quantify the rise in the number of patients diagnosed and managed in our unit and to assess patient and pregnancy outcomes.

**Materials and methods:** We undertook a review of the results of OGTTs (oral glucose tolerance tests) done in our clinic. We conducted a separate case notes review of patients with gestational diabetes who had been managed in our clinic.

**Results:** A total of 2529 OGTTs were performed between April and December 2011 in pregnant women. Out of all OGTTs performed, 254 (10.0%) were diagnostic of GDM. The number of women tested for GDM rose progressively from 215 in April to 426 in December and the number of women diagnosed each month with GDM increased in parallel from 20 in April to 41 in December. We separately identified 379 women diagnosed with GDM between November 2008 and August 2011 and managed in our unit. Most of our patients were of South Asian ethnicity (52.9%) with 27.9% being White Caucasian, 6.1% Arabic and 4.8% African or Afro-Caribbean. The average weight at the diagnosis of GDM was 82.5 kg (range 48.9 to 152.7) and the average BMI 30.2 (range 17.2 to 50.8). During pregnancy, 174 (45.9%) patients were treated by dietary changes alone, 68 (17.9%) were treated with metformin only, 99 (26.1%) with insulin only and 37 (9.8%) with both insulin and metformin. The average weight gain per week from diagnosis of GDM to delivery was 0.165 kg/week (range -0.81 to 1.26 kg/week). In women treated by diet alone, the average weight gain was 0.166 kg/week, in women

treated with metformin alone, 0.119 kg/week, in women treated with insulin alone 0.208 kg/week and in women treated with both metformin and insulin 0.160 kg/week. The average foetal weight at delivery was 3.27 kg (range 1.1–5.6 kg) and was similar in all treatment groups. Pregnancy related complications (including breech presentation, premature rupture of membranes, intrapartum foetal distress, shoulder dystocia and failure to progress in labour) were recorded in 43 (11.3%) of women. The mode of delivery was known for 369 (97.4%) women: normal vaginal delivery in 191 (68.8%) women, assisted vaginal delivery in 39 (10.6%) women and Caesarean section in 139 (37.7%). Out of the women having Caesarean sections, 54 (38.9%) had it as an emergency procedure. Six weeks postnatal OGTTs were performed in 310 (81.8%) of women. Abnormal results were found in 54 (17.4%) of women: 27 (8.4%) had impaired glucose tolerance, 10 (3.2%) had impaired fasting glycaemia, 11 (3.5%) had both impaired fasting glycaemia and impaired glucose tolerance and 6 (1.9%) had diabetes.

**Conclusion:** Out of the total number of pregnant women referred for screening, 10% have an OGTT diagnostic of GDM. After implementation of the NICE screening criteria in our area, the number of monthly OGTTs performed and GDM diagnoses has doubled in less than a year. More than half of our patients with GDM require treatment with metformin, insulin or both during pregnancy. Weight gain in pregnancy is lowest with metformin, intermediate with diet treatment or metformin-insulin combination treatment and highest in patients receiving insulin alone. Most of our patients with GDM have normal vaginal deliveries but more than 1 in 10 women have a pregnancy-related complication. More than 1 in 6 women have abnormal glucose tolerance 6 weeks after delivery with almost 2% having diabetes.

## 1091

### Evaluation of the interest of switching from a systematic screening to a targeted screening of gestational diabetes in a high-risk maternity hospital

S. Jacqueminet<sup>1</sup>, C. Ciangura<sup>1</sup>, A. Dierick-Gallet<sup>1</sup>, O. Bourron<sup>1</sup>, C. Sachon<sup>1</sup>, D. Vauthier-Brouze<sup>2</sup>, J. Nizard<sup>2</sup>, M. Dommergues<sup>2</sup>, A. Hartemann<sup>1</sup>;

<sup>1</sup>Pôle Coeur-Métabolisme, <sup>2</sup>Gynécologie-Obstétrique, Groupe hospitalier Pitié-Salpêtrière, Paris, France.

**Background and aims:** Gestational diabetes (GD) has been systematically screened in our hospital's maternity for 15 years. The 2010 French Guidelines propose a targeted screening in women having at least one of the following risk factors: BMI  $\geq 25$  Kg/m<sup>2</sup>; age  $\geq 35$  years; 1st degree family history of type 2 diabetes; personal GD history; macrosomia history. Our aim was to assess the risk level of the screened population and the potential consequences of implementing a targeted screening.

**Patients and methods:** 2370 women were prospectively followed in our hospital's maternity from September 2010 to August 2011. All the women did an oral glucose tolerance test (OGTT) 75g between 24 and 28 weeks amenorrhea. Prescriptions were given by midwives and obstetricians. Women were asked to fill in an information card, concerning GD risk factors, which was attached to the prescription. The test results with the information cards were sent to the diabetologist (GD diagnostic; IADGSP 2010 criterion)

**Results:** 1782 (75%) cards with OGTT data were received; 1209 cards (68%) were fully filled in, corresponding to: (i) 557 (46%) women without any risk factors, of whom 40 (7%) had a GD; and (ii) 652 women (54%) with at least one risk factor, of whom 190 (29%) had a GD. Amongst those 652 women the risk factors identified were: 316 (48.4%) women had a BMI  $\geq 25$  Kg/m<sup>2</sup>; 328 (50.6%) had an age  $\geq 35$  years; 228 (35%) had a 1st degree family history of type 2 diabetes; 47 (7.2%) had personal GD history; 39 (6%) had a macrosomia history in a previous pregnancy. Out of the 230 women with GD 17% had no risk factors and 83% had at least one risk factor.

**Conclusion:** Our population had a strong prevalence of GD risk factors. After discussion with the maternity team we decided to maintain systematic screening of GD. The procedure, which has been set up between the maternity and our department, is now efficient. This procedure enabled a low rate of macrosomia (5%) to be obtained as shown by a recent data analysis. A change in practice could disorganize our collaboration with the maternity, resulting in a loss of completeness in the screening of gestational diabetes in women at risk. Moreover, the percentage of women without any risk factors yet having GD is not negligible.



## 1092

### Evaluation of the results of a universal GDM screening according to both WHO and IADPSG criteria

A. Kun<sup>1</sup>, A.G. Tabak<sup>2,3</sup>, Z. Sudar<sup>4</sup>, J. Tornoczy<sup>5</sup>, P. Vargha<sup>6</sup>, Z. Kerenyi<sup>7,8</sup>, G. Tamas<sup>2,8</sup>,

<sup>1</sup>Department Obstetrics and Gynecology, Tolna County Balassa Janos Hospital, Szekszard, Hungary, <sup>2</sup>1st Department of Medicine, Semmelweis University, Budapest, Hungary, <sup>3</sup>Department of Epidemiology and Public Health, University College London, UK, <sup>4</sup>3rd Department Internal Medicine, Tolna County Balassa Janos Hospital, Szekszard, Hungary, <sup>5</sup>Diabetes Care Outpatient Unit, Tolna County Balassa Janos Hospital, Szekszard, Hungary, <sup>6</sup>Department of Cardiology, Semmelweis University, Budapest, Hungary, <sup>7</sup>Diabetology, Tóth Ilona Health Service, Budapest, Hungary, <sup>8</sup>National Centre for Diabetes Care, Semmelweis University, Budapest, Hungary.

**Background and aims:** Based on the HAPO study results, IADPSG recommended new diagnostic criteria for diabetes (GDM) in 2010. To better understand risk factors and describe the frequency of GDM a universal screening was performed using the WHO criteria in 3 representative regions of Hungary in 2007–2009. While the prevalence of GDM significantly increased in the multinational HAPO study, no similar estimates are available regarding the epidemiology of GDM in Hungary.

**Materials and methods:** Between 15th May 2010 and 14th May 2011 all pregnant women in the Szekszárd region (South West Hungary) were invited for a 3-point 2-hour oral glucose tolerance test (fasting glucose, 1-h and 2-h glucose). Results were evaluated according to both the WHO and the IADPSG criteria, while intervention was initiated only in women diagnosed by the WHO recommendation.

**Results:** Among 1080 pregnant women (age:  $29.6 \pm 5.4$  yrs [mean  $\pm$  SD], BMI at booking:  $25.6 \pm 6.0$  kg/m<sup>2</sup>) screened, GDM was found in 81 (7.5%) cases based on the WHO criteria and in 177 women (16.4%) based on the IADPSG criteria. GDM women irrespective of the diagnostic criteria used, were older ( $31.4 \pm 5.5$  vs.  $29.4 \pm 5.4$  yrs [WHO];  $P=0.0016$ ;  $30.9 \pm 5.3$  vs.  $29.4 \pm 5.4$  yrs [IADPSG];  $P=0.0004$ ), had higher systolic blood pressure ( $124 \pm 10$  vs.  $121 \pm 11$  mmHg; [WHO];  $P=0.028$ ;  $123 \pm 11$  vs.  $121 \pm 11$  mmHg [IADPSG];  $P=0.021$ ). Only 55 women were diagnosed by both diagnostic criteria with GDM. According to the IADPSG criteria 26 of the WHO-GDM cases had normal glucose tolerance and 122 additional IADPSG-GDM cases were diagnosed that were normoglycemic based on the WHO criteria. The WHO-GDM only and the IADPSG-GDM only cases had similar age ( $P>0.05$ ) however the latter group had a higher BMI ( $24.9 \pm 4.7$  vs.  $27.6 \pm 5.3$  kg/m<sup>2</sup>;  $P=0.016$ ). Caesarean section rates were similar in all groups such as the frequency of intrauterine deaths and congenital malformations. Babies born to IADPSG-GDM only mothers (without any intervention) were heavier compared to that of healthy women ( $3459 \pm 541$  vs.  $3327 \pm 502$  g;  $P=0.018$ ).

**Conclusion:** According to our results, it is expected that the frequency of GDM would double if the new IADPSG criteria were utilized in Hungary. While the increased birth weight of the IADPSG-only women supports the use of the new diagnostic criteria, the application of the IADPSG diagnostic criteria certainly requires substantial changes of the current care of pregnant women.

## 1093

### Screening of gestational diabetes in Italy: searching for the best way to diagnose it

E. Lacaria<sup>1</sup>, G. Di Cianni<sup>2</sup>, V. Resi<sup>1</sup>, A. Ghio<sup>1</sup>, C. Lencioni<sup>2</sup>, C. Goretti<sup>2</sup>, C. Sannino<sup>2</sup>, S. Del Prato<sup>1</sup>, A. Bertolotto<sup>1</sup>;

<sup>1</sup>Department of Endocrinology and Metabolism, Section of Diabetes and Metabolic Diseases, University of Pisa, <sup>2</sup>Department of Diabetes and Metabolic Diseases, Hospital of Livorno ASL6, Livorno, Italy.

**Background and aims:** The debate regarding the utility of universal screening for diagnosis of gestational diabetes (GDM) is real and continues to generate lively discussion, especially regarding expenditure of health resources. In Italy, universal GDM screening was based on Carpenter and Coustan's criteria till March 2010, when the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations were adopted leading to an immediate increase in GDM diagnosis. This increase has generated some alarm and in September 2011 the Italian Public Health Ministry recommended GDM screening only for women with risk factors. The aim of the present study was to compare GDM prevalence calculated according to different diagnostic procedures in the Italian population.

**Materials and methods:** We compared two groups of Caucasian pregnant women from the Tuscany region who have been screened for GDM according to different diagnostic criteria. Group A consisted of 3950 women undergoing universal screening based on Carpenter and Coustan's criteria during the years 2001–2003. Group B included 2274 women undergoing universal screening according to the IADPSG criteria in the years 2010–2011. The two groups were comparable for clinical and anthropometric features. Finally we have retrospectively re-evaluated GDM prevalence applying the IADPSG criteria only on Group B women deemed at GDM risk because of personal history of GDM; pre-pregnancy BMI  $\geq 25$  Kg/m<sup>2</sup>; fasting plasma glucose at the first visit 100–125 mg/dl; age  $\geq 35$  years; previous macrosomia; family history of diabetes.

**Results:** As compared to Carpenter and Coustan's, universal screening by IADPSG criteria resulted in a significant increase of the prevalence of GDM ( $P<0.04$ ). Applying IADPSG criteria only in women deemed at risk GDM marginally reduced the calculated prevalence of GDM, which remained ( $p<0.09$ ) higher as compared to the one obtained with Carpenter and Coustan's criteria.

**Conclusion:** Adoption of the IADPSG criteria is associated with a significant increase in GDM prevalence. Limiting screening in high risk women still results in significantly higher GDM prevalence. Therefore, recommendations not to test low-risk group women, in order to limit costs for the Public Health System, are dubious and require further examination.

\*In Group B (n 2274) we have identified 1887 women (83%) with at least one risk factor for GDM

	Universal Screening 2001–2003	Universal Screening 2010–2011	Selective Screening 2010–2011
Type of screening	Carpenter and Coustan GCT (50g)+OGTT(100g)	IADPSG FPG $\geq 92$ mg/dl or Ministry OGTT(75g)	Italian Health Ministry OGTT(75g)
Number	3950 (Group A)	2274 (Group B)	1887* (Group B)
Gestational Diabetes %	8.7%	18.9%	17.2%
Impaired Glucose Tolerance %	6.7%	Category not provided	Category not provided

## 1094

### Evaluation of the French guidelines for a selective screening of gestational diabetes mellitus from a cohort of 18770 women over 9 years

E. Cosson<sup>1</sup>, I. Pharisien, A. Benbara, Y. Jaber, B. Lormeau, D. Sandre-Banon, N. Assad, C. Cussac Pillegand, P. Valensi, L. Carbillon;

<sup>1</sup>AP-HP, Jean Verdier Hospital, Bondy, France

**Background and aims:** We aimed to evaluate the French societies of Diabetology and Obstetric 2010 guidelines to selectively screen for gestational diabetes mellitus (GDM), based on body mass index  $\geq 25$  kg/m<sup>2</sup> (BMI25) or age  $\geq 35$  years (age35) or family history of diabetes (FamDiab) or personal history of GDM (PrevGDM) or macrosomia (PrevMacro).

**Materials and methods:** We selected from all deliveries between 2002 and 2010 in our university hospital located in a suburb of Paris, France (n=20630,  $29.7 \pm 5.8$  years old, BMI  $24.1 \pm 4.9$  kg/m<sup>2</sup>, hypertension 2.2%, women from Europe 57%, North Africa 27%, Africa 22%) the 18.770 women with no known diabetes and for whom all risk factors (RF) were known. During this period, GDM was universally screened and defined as fasting plasma glucose level  $\geq 5.3$  mmol/l and/or 2 hour post-load (75g) glucose level  $\geq 7.8$  mmol/l.

**Results:** The prevalence of at least 1 risk factor (" $\geq 1$ RF") increased between 2002 and 2010 (51.7 to 61.5%,  $p<0.001$ ) with no change in GDM prevalence (mean 14.8%, intention to screen). Each RF was significantly associated with GDM, with odds ratio ranging from 1.1 (BMI25, age35, FamDiab) to 1.25 (PrevGDM) and 5.9 (PrevMacro); and the odds ratio for " $\geq 1$ RF" was 1.4 [95%CI 1.3–1.5],  $p<0.001$ ). Overall, 58.5% of women had  $\geq 1$ RF and would have been screened if the guidelines had been followed; and 34.7% of the GDM would have been missed. The presence of " $\geq 1$ RF" added prognostic value to the presence of GDM for new onset of hypertension or preeclampsia, large for gestational age, need for hospitalization during pregnancy and elective caesarean section ( $p<0.001$  for all).

**Conclusion:** The presence of  $\geq 1$ RF is predictive of GDM and its prevalence increases. However, the sensitivity of selective screening is 65.3%. Although the women with both GDM and  $\geq 1$ RF were more often hospitalized and had more elective caesarean sections, they had more preeclampsia and large for

gestational age babies. Therefore, selective screening as recommended in France is not sensitive enough, but detects the women with the highest risk of GDM complications. These data remain to be checked using the new diagnostic criteria.

## 1095

### The effect of gestational diabetes mellitus (GDM) on maternity care and costs in Ireland

P. Gillespie<sup>1</sup>, C. O'Neill<sup>1</sup>, J. Cullinan<sup>1</sup>, F. Dunne<sup>2</sup>;

<sup>1</sup>School of Business and Economics, <sup>2</sup>School of Medicine, Galway, Ireland.

**Background and aims:** Given the increasing prevalence of GDM, as well as its potentially serious implications for maternal and neonatal outcomes, a literature has emerged which examines the economic impacts of GDM. This study explores the independent effects of GDM on maternity care and costs in Ireland, over and above the effects of other potentially important risk factors.

**Materials and materials:** Prospective analysis of 4372 women in the Irish ATLANTIC DIP Study between 2007 and 2009 who had a 75-g oral glucose tolerance test at 24–28 weeks during their pregnancy. Mode of delivery was recorded as normal vaginal delivery (NVD), assisted vaginal delivery (forceps and/or ventouse) (AVD), elective caesarean section (ELCS), and emergency caesarean section delivery (ERCS). Neonatal unit admission was recorded as whether or not an infant was admitted for neonatal intensive care. Unit cost estimates (Euros in 2010 prices) were applied to value resource use (€2417 per NVD case, €3599 per AVD case, €6033 per ELCS case, €7518 per ERCS, and €7528 per neonatal admission). Multinomial logistic, logistic, and generalised linear model multivariate regression analysis was used to explore the effects of GDM and other independent variables (age, body mass index, primiparous, family history of diabetes, previous miscarriage, ethnicity, and delivery week) on three dependent variables: mode of delivery, neonatal unit admission, and costs.

**Results:** Using the International Association of Diabetes and Pregnancy Study Group criteria (fasting blood glucose >5.1 mmol/l, 1-h value >10 mmol/l, and 2-h value >8.5 mmol/l), a total of 354 women or 8.1% had a positive GDM diagnosis. Mode of delivery for non-GDM cases consisted of 64.9% for NVD, 18.3% for AVD, 8.5% for ELCS, and 8.4% for ERCS. This compared to 59.5%, 15.1%, 13.7% and 11.8% respectively for GDM cases. After controlling for other factors, the odds ratio for an ERCS relative to a NVD was 1.75 (95% CIs: 1.08, 2.81,  $P < 0.05$ ) for GDM relative to non-GDM cases. Neonatal unit admission for non-GDM cases was 10.42% compared to 28.70% for GDM cases. After controlling for other factors, the odds ratio for a neonatal admission was 3.14 (95% CIs: 2.27, 4.34,  $P < 0.01$ ) for GDM relative to non-GDM cases. The mean cost per non-GDM case was €4117 (SD: 3019) compared to €6039 (SD: 4378) per GDM case. After controlling for other factors, the incremental cost per GDM case was €1597 (95% CIs: 1086, 2108,  $P < 0.01$ ).

**Conclusion:** GDM was associated with significant effects on maternity care and costs over and above the effects of other potentially important factors including overweight or obesity during pregnancy. In particular, GDM was associated with higher levels of emergency caesarean section and neonatal unit admission, which translated into higher maternity care costs overall.

Variable /Model	Model 1 Mode of Delivery				Model 2 Neonatal Admission	Model 3 Total cost (€)
	Odds Ratios (SE)				Odds Ratios (SE)	Beta (SE)
	NVD (reference)	AVD	ELCS	ERCS		
GDM	—	1.15 (0.28)	1.18 (0.28)	1.75 (0.43)**	3.14 (0.52)***	1597 (261)***
Log Likelihood		2887.92			-1124.83	-30451.58

Note 1: NVD = normal vaginal delivery; AVD = assisted vaginal delivery; ELCS = elective caesarean section; ERCS = emergency CS

Note 2: \* =  $p < 0.10$ ; \*\* =  $p < 0.05$ ; \*\*\* =  $p < 0.01$

Note 3: Model 1 - multinomial Logistic; Model 2 - Logistic; Model 3 - Generalised Linear Model (Gamma variance/identity link)

Note 4: All models estimated controlling for BMI, age, parity, family, history, ethnicity, previous miscarriage, delivery week

Supported by: Health Research Board in Ireland

## 1096

### The diagnosis of gestational diabetes mellitus in women submitted to Roux-in-Y gastric bypass

C. Freitas, M. Monteiro, G. Rocha, D. Seabra, M. Nora, C. Araújo;

Centro Hospitalar Entre Douro e Vouga, Santa Maria da Feira, Portugal.

**Background and aims:** Surgical treatment of severe obesity is growing worldwide and Roux-en-Y gastric bypass is one of the most common bariatric surgeries performed. Almost half of all the patients submitted to this treatment, are women in fertile age. Several series of well succeeded pregnancies have been published. Overall, there are few serious complications and the classical obesity related comorbidities seem to improve, including the incidence of Gestational Diabetes Mellitus (GDM). Following the recent recommendations of the International Association of Diabetes and Pregnancy Study Groups, the new criteria for diagnosis GDM were adopted in January of 2011 in Portugal. Until 2011, women were screened with the O'Sullivan test. When positive, they performed a 100 gr Oral Glucose Tolerance Test (OGTT) and needed to have 2 altered values to establish the diagnosis. After January of 2011, pregnant women are screened in the first trimester with a fasting glucose value, or between 24–28 weeks of gestation, with a 75 gr OGTT, in which only one glucose value of is necessary to fulfill the GDM diagnosis criteria. One of the aims of these criteria is to uniform the diagnosis. Nevertheless, people who have been submitted to gastric surgery shouldn't perform a OGTT. The authors intended to compare the prevalence of GDM in pregnant women submitted to gastric bypass before and after the introduction of these universal diagnosis criteria.

**Materials and methods:** We conducted a retrospective study based on the clinical data of all the pregnant women submitted to gastric bypass referred to the Obstetric outpatient clinic between 2004 and 2011.

**Results:** There were 33 pregnancies, 18 until the end of 2010 with no diagnosis of GDM and 15 after January of 2011, including one abortion and a woman with type 1 diabetes. Applying the new diagnosis criteria to the other 13 women, there were 7 diagnosis of GDM (53.8%), all established with the 60 minutes' glucose value in the OGTT, at  $25 \pm 2.3$  weeks of gestation. Reviewing all the TTOG performed ( $n=19$ , 7 with 100 gr, 12 with 75 gr) we found the same glycemic profile: in the first group glycemic values of 70,8 - 183,5 - 80,3 - 58,8 gr/dl at 0 - 60 - 120 - 180 minutes; in the second values of 71,5 - 173 - 59,3 mg/dl at 0 - 60 - 120 minutes respectively. Eleven of the women who performed a TTOG experimented an hypoglycemia (57,9%). All of the pregnant women with GDM had an excellent metabolic control, despite 2 of them needing 8 UI of intermediate acting insulin at bedtime. Comparing the outcome of these pregnancies and the ones of the women who didn't have GDM, we didn't find any statistical difference. Nevertheless the first ones tended to be older at the time of the gastric bypass (31 vs 28,6 years) and the pregnancy (33,3 vs 30,4 years). Despite having passed more time since the surgery (27,7 vs 19,9 months), women with GDM lost less weight (27,8 vs 40,4 Kg) and had higher Body Mass Index (BMI) at the onset of the pregnancy (30,4 vs 27,4 Kg/m<sup>2</sup>). There were no differences in the outcomes of the pregnancies between the 2 groups.

**Conclusion:** The new criteria for diagnosis of GDM can't be applied to women submitted to gastric bypass for 2 main reasons: first the absorption of an oral glucose load is altered after a gastric surgery and thus, the recommended values can't be applied; second, there is an unacceptable incidence of hypoglycemia directly caused by that glucose load. More studies are needed to support some evidence to apply other tests for the diagnosis of GDM in these women.

## PS 094 Lessons from microvascular cohort studies

### 1097

#### Prevalence and correlates of complicated diabetes mellitus and associated co-morbidities among Sri Lankan adults: the Sri Lanka diabetes and cardiovascular study

P. Ranasinghe<sup>1</sup>, R. Jayawardena<sup>2</sup>, G.R. Constantine<sup>2</sup>, M.H.R. Sheriff<sup>2</sup>, D.R. Matthews<sup>3</sup>, P. Katulanda<sup>2</sup>;

<sup>1</sup>Department of Pharmacology, Faculty of Medicine, Colombo, Sri Lanka,

<sup>2</sup>Diabetes Research Unit, Department of Clinical Medicine, Faculty of Medicine, Colombo, Sri Lanka, <sup>3</sup>Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, UK.

**Background and aims:** Diabetes is a leading cause of morbidity and mortality in the world. South Asians are known to be at a higher risk of developing diabetes and have differential risk factors. They are also known to have an earlier onset and a more aggressive progression of the disease than other ethnic groups. We evaluated the prevalence and correlates of microvascular complications and other cardio-metabolic co-morbidities among Sri Lankan adults with diabetes.

**Materials and methods:** This community-based cross-sectional national study on diabetes was conducted in seven of the nine provinces in Sri Lanka between years 2005–2006. Diabetes was diagnosed according to the American Diabetes Association and World Health Organization criteria. Presence/absence of diabetic retinopathy was determined by standardized expert ophthalmological examination. Nephropathy was defined as the presence of micro-/macro- albuminuria, or by perusal of medical records. Neuropathy was diagnosed according to the validated Toronto Clinical Scoring System. Data on physical activity were collected using the short version of the International Physical Activity Questionnaire. Metabolic syndrome was diagnosed based on International Diabetic Federation criteria. Abdominal obesity was defined as waist circumference (WC) > 90 cm in males and > 80 cm in females. Data were analysed using SPSS v15, in all analyses a  $p < 0.05$  was considered statistically significant.

**Results:** Sample size was 4,485 (Response rate-89.7%), 39.5% ( $n=1,772$ ) were males and 17.6% ( $n=789$ ) were residing in urban areas. Mean age was  $46.1 \pm 15.1$  years. The age- and sex-adjusted prevalence of Diabetes, Impaired Glucose Tolerance and Impaired Fasting Glucose were 10.3% (males:9.8%, females:10.9%,  $p < 0.001$ ), 5.4% (males:4.2%, females:6.5%) and 4.4% (males:5.6%, females:3.1%) respectively. The prevalence of micro-vascular complications in diabetic patients were; retinopathy-24.1% (males:27.6%, females:22.6%), nephropathy-4.3% (males:4.1%, females:4.3%) and neuropathy-44.8% (males:44.0%, females:45.2%). Majority had at least one complication (47.2%), 28.6% had two complications, while 20.9% had none of the microvascular complications. In those with diabetes, central obesity was present in 53.2% (males:40.0%, females:61.0%,  $p < 0.001$ ). Prevalence of Metabolic Syndrome was 73.3% (males:64.0%, female:78.9%,  $p < 0.001$ ), while hypertension was present in 55.0% (males:55.3%, females:55.1%). There were no significant differences observed in mean values of weight, BMI, waist circumference, hip circumference, WHR, BP, FBG, 2-hr PPBS, LDL, HDL and total cholesterol in those with and without microvascular complications. In diabetic patients with co-morbid hypertension, metabolic syndrome or obesity the mean FBG, 2-hr PPBS, BMI, waist circumference, systolic BP and diastolic BP was higher than those without co-morbidities.

**Conclusion:** Diabetes is common among Sri Lankan adults, with a high prevalence of microvascular complications and associated co-morbidities. There is an urgent necessity for public health and clinical interventions to tackle the ongoing epidemic.

Supported by: National Science Foundation, Sri Lanka

### 1098

#### Development and progression of diabetes related co-morbidities in patients with type 1 diabetes: the JEVIN trial - a 20 year follow-up

T. Heller<sup>1</sup>, N. Müller<sup>1</sup>, C. Kloos<sup>1</sup>, R. Schiel<sup>2</sup>, I.S. Ross<sup>3</sup>, G. Wolf<sup>1</sup>, U.A. Müller<sup>1</sup>;

<sup>1</sup>Internal Medicine III, University Hospital Jena, Germany, <sup>2</sup>Clinic for Diabetes and Metabolic Diseases, MEDIGREIF Inselklinik Heringsdorf, Germany, <sup>3</sup>University of Aberdeen, UK.

**Background and aims:** In 1989/90 the JEVIN-Trial started as a cross-sectional survey on the quality of diabetes care of insulin treated patients with diabetes type 1 and 2 ( $n=190$ ; age 16–60y). Every 5 years, the cohort was analysed. In 2004/05 and 2009/10 only patients with type-1-diabetes were examined. Since the beginning of the trial enormous changes in social structure and medical care and therapy have occurred. The health care system was decentralised in 1989/90 and structured treatment, as well as teaching programmes for intensified insulin therapy were established. Since 1995, when privately run specialised diabetes out-patient care was established, human insulin replaced animal insulin, insulin analogues were introduced, insulin pump therapy was implemented and multiple daily blood glucose controls became standard. The aim of the investigation was to detect the development and progression of retinopathy, nephropathy and peripheral neuropathy.

**Materials and methods:** Of 131 patients with type 1 diabetes analysed in 1989/90, 104 (79.4%) patients were still alive in 2009/10. Of the living population 83 (79.8%) patients could be identified and of these 73 (88%) patients were examined: women 33%, age 58.5y, diabetes duration 35.2y (20–68), BMI 26.64kg/m<sup>2</sup>, blood pressure 143/82mmHg, HbA<sub>1c</sub> 6.9 (HbA<sub>1c</sub> is DCCT adj.).

**Results:** Seventy percent ( $n=51$ ) of the 73 patients with 20y follow up had no retinopathy at baseline. In 2009/10 still 27% ( $n=20$ ) remains having no retinopathy whereas 51% ( $n=37$ ) had non-proliferative and 18% ( $n=13$ ) proliferative retinopathy. At last observation 32% ( $n=17$ ) had retinal laser treatment. HbA<sub>1c</sub> in patients without/with retinopathy was 7.3%/7.6% (56.3mmol/mol / 59.6mmol/mol; n.s.) at baseline and 6.5%/7.0% (47.5mmol/mol / 53.1mmol/mol; n.s.) at follow up. At baseline 86% ( $n=63$ ) of the patients were free of neuropathy compared to 56% ( $n=41$ ) at follow up. Diabetic foot syndrome (ulcer/gangrene) was prevalent in 3 patients at baseline and in 3 patients at follow up. No amputations were present at baseline but in 2 patients at follow up. HbA<sub>1c</sub> in patients without/with neuropathy was 7.3%/8.1% (56.3mmol/mol / 65.0mmol/mol; n.s.) at baseline and 6.7%/7.1% (49.7mmol/mol / 54.1mmol/mol; n.s.) at follow up. Tests of sensibility were without pathological findings in 45% ( $n=33$ ) and the peripheral pulse status in 70% ( $n=51$ ). No diabetes related nephropathy was present in 77% ( $n=56$ ) in 1989/90 and 49% ( $n=36$ ) at follow up. HbA<sub>1c</sub> in patients without/with nephropathy was 7.4%/7.5% (57.4mmol/mol / 58.5mmol/mol; n.s.) at baseline and 6.8%/7.1% (50.8mmol/mol / 54.1mmol/mol; n.s.) at follow up.

**Conclusion:** After a mean diabetes duration of 35 years and a follow-up of 20 years diabetes related retinopathy increased in prevalence from 30% to about 70% (40%, 2%/y.) compared to 1989/90. In the same period neuropathy increased from 14 to 66% (42%, 2%/y.) whereas the prevalence of nephropathy rose from 23% to 51% (28%, 1.4%/y.). Patients without complications had a lower HbA<sub>1c</sub> at baseline and follow up, but it was not significant. The increase in prevalence is low in comparison to the long follow-up period.

### 1099

#### Impact of HbA<sub>1c</sub> followed from diagnosis on retinopathy and nephropathy in patients with onset of type 1 diabetes before 35 years

H.J. Arnqvist<sup>1</sup>, M. Nordwall<sup>2</sup>, M. Fredriksson<sup>3</sup>, M. Dhir<sup>4</sup>, M. Abrahamsson<sup>4</sup>, J. Ludvigsson<sup>2</sup>, The VISS study group;

<sup>1</sup>Diabetes and Endocrinology, Clinical and experimental medicine,

<sup>2</sup>Pediatrics, <sup>3</sup>Linköping Academic Research Centre, Linköping University,

<sup>4</sup>Ophthalmology, Vrinnevi Hospital, Sweden.

**Background and aims:** HbA<sub>1c</sub> was introduced in diabetes care in the early 80's. HbA<sub>1c</sub> is related to diabetic complications but it is still controversial what HbA<sub>1c</sub> level to aim for in treatment of type 1 diabetes. Our aim was to study the impact of HbA<sub>1c</sub> followed from onset of diabetes, on the incidence and prevalence of retinopathy and nephropathy in an unselected population of patients with type 1 diabetes.

**Materials and methods:** All 480 patients diagnosed with type 1 diabetes before the age of 35 years during 1983–1987 in a region of South East Sweden were followed 20–25 years. Retinopathy was evaluated by fundus photography and albuminuria/nephropathy data were collected from medical records. Long term weighted mean HbA<sub>1c</sub> was calculated.



**Results:** Only 13% had no signs of retinopathy. The prevalence of lower grades of simplex retinopathy was 33%, more severe simplex retinopathy 41% and proliferative retinopathy 13%. Long term mean HbA1c was 55, 63, 69 and 78 (range 57–103) mmol/mol in these groups. The median time to first sign of retinopathy was longer in patients with long term HbA1c  $\leq$  56 mmol/mol compared to patients with long term HbA1c  $\geq$  78 mmol/mol, 17.7 and 11.4 years, respectively ( $p < 0.001$ ). The prevalence of microalbuminuria was 11% and macroalbuminuria 3%. Long term mean HbA1c was 71 mmol/mol and 86 mmol/L (range 68–110) mmol/mol in these groups compared to 65 mmol/mol in patients with no signs of nephropathy ( $p < 0.001$ ).

**Conclusion:** There is a strong association between long term mean HbA1c from diagnosis and development of microvascular complications. Long term mean HbA1c around 55 mmol/mol or lower seems to prevent proliferative retinopathy and nephropathy.

Supported by: Barndiabetesfonden and FORSS

## 1100

### Insulin antibodies are associated with unstable plasma glucose levels and diabetic retinopathy in subjects with type 2 diabetes mellitus

K. Takahashi, M. Ono, M. Matsui, T. Sasai, T. Takahashi, H. Homma, T. Kajiura, H. Taneichi, N. Takebe, J. Satoh;  
Department of Diabetes and Metabolism, Iwate Medical University, Morioka, Japan.

**Background and aims:** The clinical significance of insulin antibodies (IA) cannot be ignored, even after the introduction of either recombinant human or analog insulin. Previous studies have suggested associations of IA with hypoglycemia and hyperglycemia. Furthermore, in a recent study, elevated fasting insulin in the presence of IA was found to be an independent risk factor for CHD in insulin-treated adults. Interpretation of these adverse events has, however, often been limited by a lack of controlled observations and small sample sizes. Herein, we endeavored to ascertain the clinical features of IA-positive type 2 diabetic subjects treated with recombinant human or analogue insulin.

**Materials and methods:** The subjects were inpatients at our university. The study groups consisted of 72 females and 117 males, with a mean age of  $57 \pm 17$  yrs, diabetes duration of  $12 \pm 9.8$  yrs, and HbA1c of  $13 \pm 2.1\%$ . IA levels were measured employing RIA kits for IA with reduced non-specific bindings (Yamasa Co, Tokyo, Japan.) This study was conducted in accordance with the Declaration of Helsinki. Differences in variables between groups were compared by the Mann-Whitney U test. Multiple linear regression analyses were performed to evaluate the combined effects of factors associated with IA.

**Results:** Forty-nine of 105 subjects (46.7%) receiving current or previous therapy with recombinant human or analogue insulin were IA positive (percent binding  $14 \pm 23\%$ ), whereas all of those without ( $n=84$ ) such therapy were IA negative. Among the subjects treated with insulin, those with IA had significantly higher daily insulin requirements ( $37 \pm 23$  vs  $28 \pm 21$  U/ day,  $P=0.043$ ), longer diabetes duration ( $17 \pm 8.8$  vs  $11 \pm 9.6$  yrs,  $P=0.001$ ), longer duration of insulin therapy ( $7.3 \pm 4.9$  vs  $2.4 \pm 4.5$  yrs,  $P=0.001$ ), lower fasting serum C-peptide (CPR) levels ( $1.2 \pm 1.3$  vs  $2.0 \pm 1.6$  ng/ml,  $P=0.024$ ) and higher M-values ( $53 \pm 60$  vs  $35 \pm 36$ ,  $P=0.041$ ), an index of plasma glucose control, than those without IA. Multiple regression analyses revealed that treatments with protaminated insulin ( $\beta=0.318$ ,  $P=0.001$ ), and with regular insulin ( $\beta=-0.259$ ,  $P=0.006$ ) significantly predicted percent binding with IA. This rate was found to be a unique explanatory variable for M-values ( $\beta=0.381$ ,  $P=0.003$ ), whereas fasting serum CPR levels were not. Interestingly, diabetic retinopathy was predicted by the percent binding with IA ( $\beta=0.255$ ,  $P=0.006$ ), but not by M-values.

**Conclusion:** The present study demonstrated that IA may cause unstable plasma glucose levels in type 2 diabetic subjects maintained on recombinant human or analogue insulin therapy. The subjects positive for IA showed significantly lower fasting CPR levels, which, however, seemed rather unlikely to lead to brittleness. Therapy with protaminated insulin was positively associated with the emergence of IA, which could be interpreted, based on a previous observation, as indicating that protaminated proteins had potentiated antigenicity. To our knowledge, this is the first report showing a positive correlation between percent binding with IA and diabetic retinopathy. The mechanism is not associated with unstable plasma glucose levels, and should be further investigated. In conclusion, we propose that the emergence of IA and its concomitant adverse effects should be taken into account, when starting insulin therapy in subjects with type 2 diabetes.

## 1101

### High regression rate of macroalbuminuria may be obtained from intensive blood pressure and blood glucose controls in type 2 diabetes: a 5-year observational cohort study

H. Yokoyama<sup>1</sup>, J. Honjo<sup>1,2</sup>, M. Okuda<sup>1</sup>, S. Kanno<sup>1</sup>, H. Sone<sup>1,3</sup>, S. Okizaki<sup>1,4</sup>, T. Moriya<sup>4</sup>, M. Haneda<sup>2</sup>;

<sup>1</sup>Internal Medicine, Jiyugaoka Clinic, Obihiro, <sup>2</sup>Asahikawa Medical University, <sup>3</sup>University of Tsukuba Institute of Clinical Medicine, Mito, <sup>4</sup>Kitasato University School of Medicine, Sagami-hara, Japan.

**Background and aims:** Individuals suffering from diabetic nephropathy are feared by a progressive increase in albuminuria with relentless decrease in glomerular filtration rate (GFR). It still remains unclear how the progression is inhibited by intensive treatments for blood pressure and blood glucose.

**Materials and methods:** Subjects were recruited consecutively from patients with type 2 diabetes who visited an outpatient clinic in 2002–2008 ( $n=2500$ ) who met the following criteria; macroalbuminuria (urinary albumin-to-creatinine ratio  $\geq 300$  mg/gCr) with eGFR  $\geq 30$  ml min<sup>-1</sup> 1.73 m<sup>-2</sup> at the first visit, and were treated for more than one year. Among the overall patients 211 subjects were eligible for this observational cohort study. The aims of treatment were SBP  $< 130$  mmHg and HbA1c (NGSP)  $< 6.9\%$ . Subjects were followed until the onset of end-stage renal disease (ESRD) or end of follow-up.

**Results:** The mean follow-up period was 4.5 years. After one-year treatment, SBP and HbA1c levels were decreased (from  $147 \pm 21$  to  $131 \pm 16$  mmHg and from  $9.17 \pm 1.11$  to  $7.46 \pm 1.34\%$ ,  $p < 0.0001$  for both, respectively). Simultaneously, the ACR and eGFR levels were decreased (from 816 (IQR 463–1530) to 391 (122–1061) mg/gCr and from  $71.9 \pm 24.9$  to  $63.7 \pm 23.7$  ml min<sup>-1</sup> 1.73 m<sup>-2</sup>,  $p < 0.0001$  for both, respectively). Percentage of subjects whose ACR regressed to  $< 300$  mg/gCr was 47% at 1-year and it persisted to 52% at 5-year. Cumulative incidences of ACR  $< 300$  and  $< 30$  mg/gCr at 5-year were 45% (95%CI 38–52%) and 12% (95%CI 7–19%). Subjects with one-year SBP and HbA1c levels lower than the median levels had more than three-times higher rate of regression to ACR  $< 300$  mg/gCr than those with both levels greater than the median levels ( $p < 0.001$ , Table). Proportion of subjects whose eGFR was preserved  $\geq 30$  ml min<sup>-1</sup> 1.73 m<sup>-2</sup> was 82% at 5-year, predominantly in subjects whose ACR regressed to  $< 300$  mg/gCr. Cumulative incidences of ESRD and ESRD plus doubling of serum creatinine at 5-year were 8% (95%CI 3–13%) and 25% (95%CI 18–32%), respectively.

**Conclusion:** It is suggested that substantial improvements in blood pressure and blood glucose control may lead to a regression of macroalbuminuria and preservation of renal function, in about 50% of those who had already been suffering from overt diabetic nephropathy.

	SBP at one-year (mmHg)	
A1C at one-year (%)	>Median (129)	$\leq$ Median SBP (129)
>Median (7.2)	77 (95%CI 44–124)	142 (95%CI 88–213)
$\leq$ Median (7.2)	182 (95%CI 118–262)	254 (95%CI 180–341)

Incidence density (per 1000 person-years) of regression from macroalbuminuria (ACR  $\geq 300$  mg/gCr) to ACR  $< 300$  mg/gCr according to one-year SBP and A1C levels in subjects with T2DM who once had macroalbuminuria on their first visits.

## 1102

### Progression of diabetic nephropathy during long term RAS blockade

G. Andrésdóttir, M. Linnemann Jensen, P. Rossing;  
Steno Diabetes Center A/S, Gentofte, Denmark.

**Background and aims:** Previous studies have shown that antihypertensive treatment has a beneficial effect on progression of diabetic nephropathy. Blocking the Renin-Angiotensin System (RAS) is thought to benefit kidney function independent of the regulation of blood pressure and has been recommended for treatment of diabetic nephropathy since 2001. However, long term effects of RAS blockade have been questioned based on register studies. Our aim was to evaluate long term decline in renal function in diabetic ne-

phropathy during the last decade, in comparison to historic data before 2000 in type 1 and type 2 diabetic patients.

**Materials and methods:** This was an observational cohort study. All patients with diabetic nephropathy at our outpatient diabetes clinic were followed with annual measurements of GFR ( $^{51}\text{Cr}$ -EDTA plasma clearance). The study included all patients with a minimum of 3 measurements during the years 2000–2010 and at least 3 years of follow up. Since 2001, RAS blocking agents have been recommended to all our patients with diabetic nephropathy. Over 95% of our cohort was prescribed RAS blocking agents for more than half of the study period. With the same method we have previously published data on decline in GFR from 1983 to 2000 for type 1 diabetes (where 59% of patients were on ACE inhibitors during more than half of the study period) and from 1983–2003 for type 2 (where 24% were prescribed RAS blocking treatment at baseline).

**Results:** 315 type 1 diabetic patients met inclusion criteria. The mean (SD) GFR at baseline was 78 (29) ml/min/1.73m<sup>2</sup>, HbA<sub>1c</sub> 9.2 (1.4) %, blood pressure 140 (17) / 79 (8) mmHg, serum cholesterol 5.3 (1.0) mmol/l and albumin excretion rate (AER) median [interquartile range] was 420 [156–945] mg/24h. Patients were followed for 8.6 [5.6–9.9] years, with 7 [5–9] measurements of GFR. The rate of decline of GFR was 3.3 (3.1) ml/min/1.73m<sup>2</sup>/year, a 19% reduction (95%CI 5–34%,  $p=0.009$ ), compared to historically 4.0 (3.4) ml/min/1.73m<sup>2</sup>/year,  $n=301$ . 286 type 2 diabetic patients were included. The mean GFR at baseline was 80 (29) ml/min/1.73m<sup>2</sup>, HbA<sub>1c</sub> 8.5 (1.6), blood pressure 149 (17) / 82 (9) mmHg, serum cholesterol 4.8 (1.3) mmol/l and AER 436 [252–890] mg/24h. Patients were followed for a median of 6.0 [4.3–8.4] years with 5 [4–7] measurements of GFR. The annual mean decline in GFR was 4.4 (4.0) ml/min/1.73m<sup>2</sup>/year, reduced with 14% (95% CI 4–28%,  $p=0.04$ ) compared to historically 5.2 (4.1) ml/min/1.73m<sup>2</sup>/year,  $n=227$ . Baseline data in the historic and present cohorts were similar although AER was slightly lower in the present cohort, and blood pressure was lower for type 2 patients. In addition diabetes duration was longer for the present type 1 cohort.

**Conclusion:** Modern treatment of diabetic nephropathy, including long term blocking of the RAS, preserves renal function better than treatment used in the past decades where RAS inhibition was not standard.

## 1103

### Usefulness of UAER and GFR combination to predict all- cause mortality in type 2 diabetic patients

A.B. Mañas - Martínez, Y. Blasco- Lamarca, B. Campos- Gutiérrez, B. García- García, J. Altemir- Trallero, J.A. Gimeno- Orna, J. Ocón- Bretón; Endocrinology, Lozano Blesa Hospital, Zaragoza, Spain.

**Background and aims:** Stages of chronic kidney disease (CKD) are currently defined mainly by estimated glomerular filtration rate (eGFR). However mortality risk depends not only on eGFR but also on urinary albumin excretion rate (UAER). Our study was aimed to evaluate a new CKD staging system based on eGFR and UAER to predict all-cause mortality in type 2 diabetic patients.

**Materials and methods:** We designed a prospective cohort study with inclusion of 453 type 2 diabetic individuals with follow-up until death or March 2011. GFR (ml/min per 1.73 m<sup>2</sup>) was estimated by using the Modification of Diet in Renal Disease Study equation, and UAER (mg) by using 24-hour urine collection. CKD staging was accomplished in accordance with a system based on the NKF guidelines (current system) and in accordance with an alternative system of CKD risk categories. The alternative system was composed of five categories: group 0: eGFR  $\geq 60$  and UAER  $< 30$ ; group 1: eGFR  $\geq 60$  and UAER 30–300 or eGFR 45–59 and UAER  $< 30$ ; group 2: eGFR 30–44 and UAER  $< 30$  or eGFR 45–59 and UAER 30–300; group 3: eGFR 30–44 and UAER 30–300 or eGFR  $\geq 60$  and UAER  $> 300$  or eGFR  $< 30$  and UAER  $< 30$ ; group 4: eGFR  $< 30$  and UAER  $> 30$  or eGFR  $< 60$  and UAER  $> 300$ . Groups 3 and 4 were grouped. Incidence rates for all-cause mortality were calculated per 1000 persons-years. Comparison of the rates was done by Kaplan-Meier analysis and the log-rank test. Age and sex adjusted Cox's regression models were used to assess the predictive power of the two evaluated systems. The difference in the  $-2 \ln(L)$  between models follows a  $\chi^2$  distribution, with a higher  $\chi^2_{LR}$  value indicating a more powerful predictor.

**Results:** We included 453 type 2 diabetic patients (39.3% males). At baseline age of the study group was 65 (SD 9.3) years and duration of diabetes was 10.5 (SD 7.5) years. UAER was  $< 30$  in 321 subjects (70.8%), 30–300 in 105 (23.2%) and  $> 300$  in 27 (6%). eGFR was  $\geq 60$  in 318 (70.2%) and  $< 60$  in 135 (29.8%). According to the current system 234 (51.7%) patients belonged to stage 0, 84 (18.5%) to stages 1–2 and 135 (29.8%) to stages  $\geq 3$ . According to the alterna-

tive system 234 (51.6%) patients were classified in group 0, 152 (33.6%) in group 1, 33 (7.3%) in group 2 and 34 (7.5%) in groups 3–4. During 11.5 years follow-up, 207 (45.7%) patients died. Incidence rates of all-cause mortality were steadily increased in the successive current system stages: 28.7/1000 in stage 0, 46.3/1000 in stages 1–2 and 57.9/1000 in stages  $\geq 3$  ( $p < 0.0001$ ). Incidence rates were also steadily increased in the successive alternative system groups: 28.7/1000 in group 0, 42.7/1000 in group 1, 74.1/1000 in group 2 and 96.8/1000 in groups 3–4 ( $p < 0.0001$ ). The overall  $\chi^2$  of the age and sex adjusted model with the inclusion of eGFR and UAER was 133.1. The overall  $\chi^2$  of the age and sex adjusted model with inclusion of current-system risk categories was 111.4. The  $\chi^2$  increased up to 127.4 when the current system was replaced by the alternative one ( $p < 0.0001$  the difference between models). In a multivariate analysis including age, sex, hypertension, smoke, lipid profile, diabetes duration, A1c and prevalent macrovascular disease the alternative system was an independent predictor of mortality risk: group 1: HR 1.08 (CI 95%: 0.77–1.52;  $p=ns$ ); group 2: HR 1.71 (CI 95%: 1.03–2.83;  $p=0.038$ ); groups 3–4: HR 2.42 (CI 95%: 1.46–4.02;  $p=0.001$ ).

**Conclusion:** An alternative system of CKD classification which takes into account both, eGFR and EAUR, is superior to the current system mainly based on eGFR.

## 1104

### Prevalence of microvascular complications in European patients with type 2 diabetes mellitus with and without renal impairment: results of a large worldwide cohort study

I. Conget<sup>1</sup>, EDGE Steering Committee, J.-B. Gruenberger<sup>2</sup>, G. Bader<sup>2</sup>; <sup>1</sup>Diabetes Unit, Endocrinology Dept., Hospital Clinic i Universitari, Barcelona, Spain, <sup>2</sup>Novartis Pharma AG, Basel, Switzerland.

**Background and Aims:** Presence of renal impairment and microvascular complications are important considerations in determining prognosis and treatment in patients with type 2 diabetes mellitus (T2DM). We present results of a sub-analysis from the European cohort of the Effectiveness of Diabetes control with vildagliptin and vildagliptin/metformin (EDGE) study which compared the effectiveness and safety of vildagliptin with other oral antidiabetic drugs (OADs) in T2DM patients inadequately controlled by monotherapy, under real life conditions.

**Materials and methods:** EDGE was a prospective, multinational, non-interventional, observational study in adult T2DM patients. Patients only became eligible after the add-on treatment was chosen by the physician based on patient need. Data were collected on patient history, metabolic control and treatment. Overall, 45868 patients (intent to treat) were enrolled in 27 countries across the world. Of the 22045 patients enrolled in Europe, 12384 (58.2%) had serum creatinine measured at baseline. GFR was estimated (eGFR) according to the Modification of Diet in Renal Disease (MDRD) formula and the cohort was divided in to two groups (with renal impairment [eGFR  $< 60$  ml/min/1.73m<sup>2</sup>] or without renal impairment [eGFR  $\geq 60$  ml/min/1.73m<sup>2</sup>]).

**Results:** At baseline, 2216 (17.9%) patients had renal impairment. Patients with renal impairment were older and had a longer duration of diabetes than those without renal impairment, while HbA1c was similar in both groups. Overall, microvascular complications (collected from patients' history) were more frequent (17.7% vs 9.7%) in the group with renal impairment as compared to those without. The renal impairment group also had a higher proportion of patients with one (13.5% vs 8.1%) complication (nephropathy or neuropathy or retinopathy), two (3.5% vs 1.4%) complications and three (0.7% vs 0.2%) complications. Overall, 124 patients had severe renal impairment (eGFR  $< 30$  ml/min/1.73m<sup>2</sup>). Metformin was the most commonly used OAD in both groups, with and without renal impairment. Of the 10812 (87.3%) patients taking metformin, 1823 (16.8%) had eGFR  $< 60$  ml/min/1.73m<sup>2</sup>.

**Conclusion:** Data from this large observational European cohort of patients with T2DM inadequately controlled by OAD monotherapy showed a far from negligible proportion with renal impairment. Patients with T2DM and renal impairment had a higher prevalence of microvascular complications and inadequate metabolic control, especially given the need for better glycaemic control in this patient population. Nearly 17% of patients using metformin monotherapy have some degree of renal impairment for which metformin is contraindicated, confirming results from earlier studies.

	eGFR <60 ml/ min/1.73m <sup>2</sup> (N=2216)	eGFR ≥60 ml/ min/1.73m <sup>2</sup> (N=10168)
Age in years, mean (SD)	68.1 (10.2)	61.0 (10.5)
T2DM duration in years, mean (SD)	7.3 (6.0)	5.9 (5.0)
HbA1c (%), mean (SD)	7.9 (1.3)	7.9 (1.3)
eGFR (MDRD), mean (SD)	50.0 (8.9)	88.1 (21.7)
1 microvascular complication, n (%)	299 (13.5)	823 (8.1)
2 microvascular complications, n (%)	78 (3.5)	142 (1.4)
3 microvascular complications, n (%)	16 (0.7)	21 (0.2)
No microvascular complication, n (%)	1823 (82.3)	9182 (90.3)
Metformin	1826 (82.4)	8986 (88.4)
Sulfonylureas	347 (15.7)	971 (9.5)
Thiazolidinediones	17 (0.8)	83 (0.8)

Supported by: Novartis Pharma

## 1105

### Defining the relationship between HbA<sub>1c</sub> and average plasma glucose in type 2 diabetes and chronic kidney disease

C. Lo<sup>1,2</sup>, M. Lui<sup>1</sup>, S. Ranasinha<sup>1</sup>, H.J. Teede<sup>1,2</sup>, P.G. Kerr<sup>3</sup>, K. Polkinghorne<sup>3</sup>, D.M. Nathan<sup>4,5</sup>, H. Zheng<sup>6,5</sup>, S. Zoungas<sup>1,7</sup>,  
<sup>1</sup>Diabetes and Cardiovascular Research Unit, Monash University, Clayton, Australia, <sup>2</sup>Diabetes and Vascular Unit, Monash Medical Centre, Clayton, Australia, <sup>3</sup>Renal Unit, Monash Medical Centre, Clayton, Australia, <sup>4</sup>Diabetes Centre, Massachusetts General Hospital, Massachusetts, USA, <sup>5</sup>Harvard Medical School, Boston, USA, <sup>6</sup>Biostatistics Centre, Massachusetts General Hospital, Boston, USA, <sup>7</sup>Diabetes Research Program, The George Institute of Public Health, Sydney, Australia.

**Background and aims:** Diabetic chronic kidney disease is a growing problem globally. Glycemic control is important for the prevention of adverse kidney outcomes. However, the utility of assessing HbA<sub>1c</sub> levels in patients with chronic kidney disease (CKD) remains unclear. Here we examine the relationship between average plasma glucose (AG) and HbA<sub>1c</sub> in patients with and without CKD (stages 3 to 5).

**Materials and methods:** 43 patients with type 2 diabetes (T2DM) and CKD (33% stage 3, 23% stage 4 and 44% stage 5) with stable glycemic control, no recent changes in glucose lowering or erythropoietin therapy and no recent blood transfusions were prospectively studied and age-matched to 104 patients without CKD randomly selected from the A1c-Derived Average Glucose study. Over a 3-month period, average plasma glucose (AG) was calculated from 7–8 point self-glucose monitoring (SGM) performed 3–5 days/week and continuous glucose monitoring (CGM) performed for 2–3 days/month. Mean HbA<sub>1c</sub> was calculated as the average of levels measured at baseline and then monthly over 3 months. The relationship between AG (calculated separately from SGM and CGM) and HbA<sub>1c</sub> was analysed using multi-variable regression models.

**Results:** The CKD and non-CKD groups were well matched for age (61 ± 4 vs. 63 ± 9.6 years) and gender (40% vs. 37% were female). Mean AG and HbA<sub>1c</sub> levels were similar (CKD vs. non-CKD, AG: 9.1 ± 2.5 vs. 8.4 ± 1.9 mmol/L and HbA<sub>1c</sub>: 7.1 ± 1.4 vs. 7.0 ± 1.0 %, both p > 0.05). A linear relationship between AG and HbA<sub>1c</sub> was observed irrespective of the presence and stage of CKD. The relationship was weaker in patients with stage 4–5 CKD (non-CKD R<sup>2</sup> 0.75, stage 3 CKD R<sup>2</sup> 0.79 and stage 4–5 CKD R<sup>2</sup> 0.34). The inclusion of erythropoietin use (EPO) into the model rendered the effect of CKD stage insignificant, suggesting that the treatment of anaemia with EPO results in a systematic underestimation of the AG derived from the HbA<sub>1c</sub>: AG (mmol/L) = 1.4(HbA<sub>1c</sub> %) + 1.1 × [EPO yes (1) or no (0)] - 1.6 (p < 0.0001). However the slope of the relationship between AG and HbA<sub>1c</sub> was not modified by EPO status. In a separate analysis of CKD patients, pre-prandial AG levels were more strongly related to HbA<sub>1c</sub> than postprandial AG levels (R<sup>2</sup><sub>pre</sub> 0.59 vs. R<sup>2</sup><sub>post</sub> 0.30, p for difference in slopes < 0.001). A sensitivity analysis replacing SGM with CGM measurements produced consistent results.

**Conclusion:** In patients with type 2 diabetes and CKD, there is a linear relationship between HbA<sub>1c</sub> and AG that was more attributable to pre-prandial rather than postprandial AG levels. Compared to non-CKD patients, substantial attenuation of this relationship is observed with stage 4–5 CKD and EPO use but not stage 3 CKD. Using the derived equation in advanced CKD will

provide both clinicians and patients with more accurate information on day to day AG levels.

Supported by: CVL grant (Pfizer), ADS Grant (Servier)

## 1106

### Similar correlations between HbA<sub>1c</sub> and fasting blood glucose level independently of renal function in elder type 2 diabetic patients in the Gerodiab cohort

J. Doucet<sup>1</sup>, B. Bauduceau<sup>2</sup>, C. Verny<sup>3</sup>, J.-P. Le Floch<sup>4</sup>, SFD-SFGG Intergroup, The Gerodiab Group;

<sup>1</sup>Rouen University Hospital, Boisguillaume, <sup>2</sup>Begin University Hospital, Saint-Mande, <sup>3</sup>Bicêtre University Hospital, Paris, <sup>4</sup>Clinique de Villecresnes, Villecresnes, France.

**Background and aims:** Gerodiab is a French observational study designed to analyse the factors associated with mortality and morbidity in type 2 diabetic patients 70 year-old and over, and especially HbA<sub>1c</sub> levels. HbA<sub>1c</sub> is considered the gold standard marker of glycemic control in diabetic patients and used to treatment adaptation. However, in patients with renal insufficiency, many factors could alter the reliability of HbA<sub>1c</sub> levels, including anemia, a decreased production of erythropoietin and changes related to dialyses. In elder patients, renal dysfunction is common and thus the interest of HbA<sub>1c</sub> can be questioned. The aim of this study was to test the interest of HbA<sub>1c</sub> as a marker of glucose control in the patients included in the Gerodiab cohort.

**Materials and methods:** The correlations between HbA<sub>1c</sub> and fasting blood glucose (FBG) were analysed both in the population as a whole, and at 3 different levels of MDRD, using Pearson's linear regression.

**Results:** At inclusion, 779 out of 987 patients (375 men and 404 women, age [mean ± SD]: 77 ± 5 yr, duration of diabetes: 18 ± 12 yr) had simultaneous MDRD computation (67.4 ± 22.7 ml/min), fasting blood glucose (7.94 ± 2.89 mmol/l) and HbA<sub>1c</sub> (7.6 ± 1.3 %) assays. Similar correlations were found in the global population: HbA<sub>1c</sub> = 5.95 + 1.13 FBG; R<sup>2</sup> = 0.194; P < 0.001, in patients with MDRD < 30 ml/min (n = 27): HbA<sub>1c</sub> = 5.76 + 1.12 FBG; R<sup>2</sup> = 0.246; P < 0.001, in patients with MDRD [30–60 ml/min (n = 271): HbA<sub>1c</sub> = 6.52 + 0.80 FBG; R<sup>2</sup> = 0.096; P < 0.001, and in those with MDRD ≥ 60 ml/min (n = 481): HbA<sub>1c</sub> = 5.73 + 1.27 FBG; R<sup>2</sup> = 0.250; P < 0.001).

**Conclusion:** These results suggest that HbA<sub>1c</sub> can be used as a reliable marker of blood glucose control in elder type 2 diabetic patients, including patients with renal dysfunction.

Supported by: Novo-Nordisk and Merck Serono



## PS 095 Treatment of microvascular complications

1107

### Transplantation of bone marrow-derived mononuclear cells improves sensory disorders in the early stage of streptozotocin-induced diabetes in rats

K. Naruse<sup>1</sup>, J. Sato<sup>2</sup>, M. Funakubo<sup>2</sup>, M. Hata<sup>3</sup>, N. Nakamura<sup>1</sup>, Y. Kobayashi<sup>1</sup>, H. Kamiya<sup>4</sup>, T. Matsubara<sup>1</sup>, J. Nakamura<sup>4</sup>;

<sup>1</sup>Department of Internal Medicine, School of Dentistry, Aichi-Gakuin University, Nagoya, <sup>2</sup>Puturistic Environmental Simulation Center, Research Institute of Environmental Medicine, Nagoya University, <sup>3</sup>Department of Removable Prosthodontics, School of Dentistry, Aichi-Gakuin University, Nagoya, <sup>4</sup>Division of Diabetes, Department of Internal Medicine, Aichi Medical University, Nagakute, Japan.

**Background and aims:** Diabetic neuropathy is the most common complication of diabetes and patients with diabetic neuropathy suffer from the abnormal peripheral sensations, such as paresthesia, allodynia, hyperalgesia, and spontaneous pain. We have previously shown that the transplantation of cultured endothelial progenitor cells or mesenchymal stem cells ameliorated diabetic neuropathy in rats. In this study, we investigated whether transplantation of freshly isolated bone marrow-derived mononuclear cells (BM-MNCs) alleviates neuropathic pain in the early stage of streptozotocin (STZ)-induced diabetic rats.

**Materials and methods:** Two weeks after STZ injection into Sprague-Dawley (SD) rats, BM-MNCs or vehicle saline were injected into the unilateral hind limb muscles. Mechanical hyperalgesia and cold allodynia in SD rats were measured as the number of foot withdrawals to von Frey hair stimulation and acetone application, respectively. Two weeks after the BM-MNC transplantation, sciatic motor nerve conduction velocity (MNCV), sensory nerve conduction velocity (SNCV), sciatic nerve blood flow (SNBF), mRNA / protein expressions and histology were assessed.

**Results:** Diabetic rats showed mechanical hyperalgesia and cold allodynia. The transplantation of BM-MNCs significantly improved these sensational abnormalities in the BM-MNC-injected side of diabetic rats. The slowed MNCV/SNCV and decreased SNBF in diabetic rats were improved in the BM-MNC-injected side (MNCV: the saline-injected side;  $36.4 \pm 2.6$  m/s, the BM-MNC-injected side;  $51.4 \pm 0.8$  m/s, SNCV: the saline-injected side;  $31.7 \pm 2.9$  m/s, the BM-MNC-injected side;  $47.5 \pm 3.6$  m/s,  $P < 0.001$  for both). BM-MNC transplantation improved the decreased mRNA expression of NT-3 and number of microvessels in the hind limb muscles. The expression of calcitonin gene-related peptide was increased in the plantar skin of diabetic rats, which was abolished by BM-MNC transplantation. There was no distinct effect of BM-MNC transplantation on the intraepidermal nerve fiber density. **Conclusion:** These results suggest that autologous transplantation of BM-MNCs could be a novel strategy for the treatment of painful diabetic neuropathy.

1108

### Effect of human dental pulp stem cell transplantation on diabetic polyneuropathy

M. Hata<sup>1</sup>, K. Naruse<sup>2</sup>, Y. Kobayashi<sup>2</sup>, N. Nakamura<sup>2</sup>, S. Ozawa<sup>1</sup>, M. Shimizu<sup>3</sup>, K. Miyazawa<sup>4</sup>, M. Omi<sup>1</sup>, H. Kamiya<sup>5</sup>, J. Nakamura<sup>5</sup>, S. Goto<sup>4</sup>, K. Kurita<sup>3</sup>, Y. Tanaka<sup>1</sup>, T. Matsubara<sup>2</sup>;

<sup>1</sup>Removable Prosthodontics, School of Dentistry Aichi-Gakuin University, <sup>2</sup>Internal Medicine, School of Dentistry Aichi-Gakuin University, <sup>3</sup>Oral and Maxillofacial Surgery, School of Dentistry Aichi-Gakuin University, <sup>4</sup>Orthodontics, School of Dentistry Aichi-Gakuin University, <sup>5</sup>Internal Medicine, Aichi Medical University School of Medicine, Nagoya, Japan.

**Background and aims:** Diabetic neuropathy is the most common complication of diabetes. We have previously reported that the transplantation of cultured endothelial progenitor cells or bone marrow-derived mesenchymal stem cells ameliorated diabetic neuropathy in rats. The critical issue of cell transplantation is how to collect cells and maintain cell functions. Human dental pulp stem cells (hDPSCs) which are a sort of mesenchymal stem cells located in the dental pulp cavity are expected as a source of regenerative medicine, since hDPSCs can be isolated from wisdom tooth extraction or premolar extraction for orthodontic reasons. The aim of this study is to evaluate the

therapeutic potential of hDPSCs transplantation on diabetic polyneuropathy in streptozotocin (STZ)-induced diabetic nude mice.

**Materials and methods:** We collected human impacted third molars from 4 adults (13–23 years of age) at our hospital. Written informed consent was obtained from each donor. Dental pulp was extracted by cutting teeth and hDPSCs were isolated by collagen digestion method and cultured as previously described. Identification of hDPSCs was analyzed by surface makers using a fluorescence activated cell sorter and differentiation capabilities into adipocytes and osteoblasts. Diabetes was induced by an intraperitoneal injection of STZ in 6 week-old BALB/cA/Jcl-nu/nu mice. Eight weeks after STZ injection, hDPSCs ( $1 \times 10^5$  cells/limb) were transplanted into unilateral hindlimb skeletal muscles of normal and diabetic mice. Saline was injected into the other side as control. Sciatic blood flow (SNBF), sciatic motor /sensory nerve conduction velocity (MNCV/SNCV) and current perception threshold (CPT) were evaluated 4 and 16 weeks after the transplantation of hDPSCs. Immunohistological analysis of hindlimb skeletal muscles was also performed at the end of the experiments.

**Results:** Flow cytometric analyses showed the positive stainings of CD29 and CD90 and negative stainings of CD34 and CD45. hDPSCs differentiated into osteoblasts and adipocytes by each induction media. Diabetic mice showed significant reductions in SNBF, MNCV and SNCV and increase in CPTs in the control side compared with normal mice. Transplantation of hDPSCs significantly ameliorated the impaired SNBF, MNCV, SNCV and CPTs in the hDPSCs-injected side of diabetic mice. Immunohistological study revealed that the transplanted hDPSCs were located around the muscle bundles and not differentiated into adipocytes nor osteoblasts.

**Conclusion:** We have demonstrated the effects of hDPSC transplantation for diabetic neuropathy without any adverse effects, suggesting that the transplantation of hDPSCs could be a new strategy for the treatment of diabetic neuropathy.

1109

### Atorvastatin improves baroreflex sensitivity in patients with type 2 diabetes and dyslipidaemia

P. Grigoropoulos<sup>1</sup>, I. Eleftheriadou<sup>1</sup>, C. Zoupas<sup>1</sup>, A. Tsiakou<sup>1</sup>, S. Kalopita<sup>1</sup>, A. Kokkinos<sup>1</sup>, D. Perrea<sup>2</sup>, N. Katsilambros<sup>1</sup>, N. Tentolouris<sup>1</sup>;

<sup>1</sup>1st Department of Propaeudetic Medicine, Athens University Medical School, Laiko Hospital, <sup>2</sup>Laboratory of Experimental Surgery and Surgical Research, Athens University Medical School, Greece.

**Background and aims:** Baroreflex sensitivity (BRS) is reduced in patients with type 2 diabetes mellitus (T2DM), even in the absence of clinically apparent autonomic neuropathy. Reduced BRS has been associated with fatal ventricular arrhythmias and impaired regulation of blood pressure. Statins improve endothelial function and have been shown to have a neuroprotective effect. Therapy with statins improved BRS in subjects with hypercholesterolaemia without diabetes. However, no data exist on the effect of statins on BRS in individuals with T2DM. Aim of this study was to examine the effect of one year administration of low dose of atorvastatin on BRS in subjects with T2DM.

**Materials and methods:** A total of 82 patients with T2DM and dyslipidemia were recruited. The main inclusion criteria were age between 40–75 years, low density lipoprotein cholesterol (LDLc)  $\geq 100$  mg/dl. The main exclusive criteria were the presence of clinically apparent macrovascular disease and use of medications with known effect on autonomous nervous system activity. Forty-six patients (16 men/30 women, mean age  $60.37 \pm 8.53$ ) were assigned to atorvastatin (10 mg per day) and low-fat diet, and 36 patients (15 men/20 women, mean age  $59.06 \pm 9.23$ ) to low-fat diet only. Both groups were comparable regarding age, diabetes duration, baseline serum lipids, HbA1c, arterial blood pressure and BRS values. BRS was measured by the sequence method using the BaroCor system (Atcor Medical, Australia). All patients underwent 3 more visits, at 3, 6 and 12 months, during which the same blood tests and BRS estimation were repeated.

**Results:** Atorvastatin significantly reduced total cholesterol by 27.22% (from  $235.48 \pm 28.27$  mg/dl to  $171.41 \pm 21.33$  mg/dl,  $p < 0.001$ ), LDLc by 37.07% (from  $154.93 \pm 26.40$  mg/dl to  $97.50 \pm 22.29$  mg/dl,  $p < 0.001$ ) and TG by 19.10% (from  $144.26 \pm 70.35$  mg/dl to  $116.70 \pm 54.38$  mg/dl,  $p = 0.001$ ) after 12 months; HDL cholesterol (HDLc) remained unaffected ( $p = 0.53$ ). In the diet-treated group, no significant improvement of lipids was achieved. BRS was significantly improved in atorvastatin treated patients after 12 months therapy from  $6.46 \pm 2.79$  msec/mmHg to  $8.05 \pm 4.28$  msec/mmHg ( $p = 0.036$ ). Diet had no influence on BRS values. No difference in BMI, arterial blood pressure, heart rate, HbA1c, and fasting glucose was observed at both groups compared with baseline.

**Conclusion:** Long-term treatment with even a low dose of atorvastatin, beyond the beneficial effects on plasma lipids, improves BRS in patients with T2DM and dyslipidaemia.

## 1110

### Pharmacokinetics, safety, and tolerability of a long-acting C-peptide (CBX129801) in patients with type 1 diabetes

H. Foyt, M. Daniels, M. Milad, J. Wahren;  
Cebix Inc, La Jolla, USA.

**Background and aims:** C-peptide is co-secreted in equimolar amounts with insulin in response to elevations in blood glucose. Accumulating data suggest that C-peptide is a bioactive peptide in its own right and that its absence in type 1 diabetes patients is an important contributing factor to the onset and progression of long-term complications. In vitro studies have shown that C-peptide stimulates  $\text{Na}^+/\text{K}^+$ -ATPase and endothelial nitric oxide synthase (eNOS) and increases the expression of transcription factors important for cellular growth and cytoprotective and anti-inflammatory processes. Administration of C-peptide in type 1 diabetes patients with early stage neuropathy or nephropathy has demonstrated beneficial effects on peripheral and autonomic nerve function as well as renal function. However, efforts to replace circulating levels of C-peptide in type 1 diabetes patients have been stymied by its short biological half-life (~1 h), thus requiring multiple injections per day to mimic physiological exposure. A long-acting C-peptide CBX129801 has been developed for subcutaneous (SC) delivery following PEGylation of the peptide's N-terminus, distal from the biologically active C-terminus.

**Materials and methods:** A randomized, blinded, placebo-controlled, serial-cohort, multiple ascending dose Phase 1 study with CBX129801 has been conducted. Thirty type 1 diabetes patients (10 subjects per cohort; 8 active/2 placebo) were dosed SC at one of three dose levels: 0.3 mg, 1.0 mg (estimated C-peptide "replacement" dose level), and 3.3 mg for up to four weekly doses. Assessments of the safety, tolerability, and the single and multiple dose pharmacokinetics (PK) of plasma CBX129801 were conducted.

**Results:** Peak concentrations ( $C_{\max}$ ) generally occurred 2–4 days after SC administration.  $C_{\max}$  and AUC increased in direct proportion to increasing dose. Single dose PK was predictive of multiple dose PK. Following multiple doses of 0.3, 1.0, and 3.3 mg per week, the corresponding geometric mean  $C_{\max}$  values were 0.69, 2.3, and 10.8 nM; AUC<sub>tau</sub> values were 4.2, 14.2, and 64.5 nM·day; and  $C_{\min}$  values were 0.46, 1.60 and 6.54 nM, respectively. The mean plasma  $t_{1/2}$  of CBX129801 was 6–7 days, representing a 150-fold increase in the  $t_{1/2}$  relative to native C-peptide. CBX129801 was safe and well tolerated with no SAEs reported. The PK data from these 3 cohorts were compiled and modeled, and plasma concentration-time profiles were simulated. Simulations showed that a maintenance dose of 0.8 mg administered weekly will yield the targeted 1 nM trough plasma concentration in ~95% of patients, which represents physiologic replacement of C-peptide. This maintenance dose (0.8 mg/week) was utilized in a follow-up Phase 2 study enrolling 40 patients with SC dosing throughout the first 12 weeks with study duration of 15 weeks. Pharmacodynamic data were collected from both studies at baseline, 6 weeks, and 12 weeks in the form of electrophysiological measurements, including sural sensory nerve conduction velocities (SNCV). An exploratory responder analysis from the Phase 1 study, defined as an improvement in SNCV of >0.75 m/sec above baseline for the sural nerve in both legs, showed a favorable response for the CBX129801-treated patients relative to placebo; Phase 2 SNCV data are pending.

**Conclusion:** It is concluded that CBX129801 is safe and well tolerated in type 1 diabetes patients, and the  $t_{1/2}$  supports weekly administration. CBX129801 is a potentially attractive therapeutic agent that warrants additional testing in larger clinical trials.

Clinical Trial Registration Number: NCT 01293461

## 1111

### HM-VN118 inhibits VEGF-induced retinal vascular leakage and hypoxia-induced neovascularisation through reduced matrix metalloproteinase activity

J. Kim, C.-S. Kim, Y.M. Lee, E. Sohn, K. Jo, S.S. Dam, J.S. Kim;  
Traditional Korean Medicine (TKM) Based Herbal Drug Research Group,  
Korea Institute of Oriental Medicine, Daejeon, Republic of Korea.

**Background and aims:** Diabetes alters the structure and function of retinal vasculature, including the alteration of the blood-retinal barrier (BRB) and

the formation of retinal neovascularization. The aim of this study was to investigate the potential preventive effect of HM-VN118, an ethanolic extract of *Homonoia riparia* Lour., on VEGF-induced retinal vascular leakage and hypoxia-induced neovascularization in vivo.

**Materials and methods:** HM-VN118 was injected intraperitoneally in a rat model of VEGF-induced retinal vascular permeability and a mouse model of oxygen-induced retinopathy (OIR). BRB permeability was quantified using fluorescein angiography. Retinal angiogenesis was assessed by whole mount immunofluorescence staining of retinas. Matrix metalloproteinases (MMP) activity and tight junction protein were examined in retinal protein lysates and whole mount retinas

**Results:** When normoglycemic rats were intravitreally injected with VEGF, there was widespread leakage of fluorescein from the retinal vasculature when compared to control retinas. Treatment with HM-VN118 was shown to reduce VEGF-induced permeability and the loss of tight junction protein. Retinal neovascularization in the OIR model was significantly inhibited by HM-VN118. In addition, increases of both activity and expression of MMP-2 and MMP-9 was attenuated by the treatment of HM-VN118 in both animal models.

**Conclusion:** These results suggest that MMPs are involved in the process of VEGF-induced vascular leakage and hypoxia-induced retinal neovascularization and HM-VN118 might have therapeutic potential for the treatment of diabetic retinopathy.

Supported by: K12040

## 1112

### Topical administration of somatostatin prevents retinal neurodegeneration in experimental diabetes

C. Hernandez<sup>1</sup>, M. Garcia-Ramirez<sup>1</sup>, L. Corraliza<sup>1</sup>, A. Ciudin<sup>1</sup>, B. Ponsati<sup>2</sup>, J. Fernández-Carneado<sup>2</sup>, J. Farrera-Sinfreu<sup>2</sup>, R. Simó<sup>1</sup>;  
<sup>1</sup>CIBERDEM and Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute, <sup>2</sup>BCN Peptides, S.A., Barcelona, Spain.

**Introduction/aim:** There is growing evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy (DR). Consequently, neuroprotective drugs could open up a new strategy for the treatment of the early stages of DR. Somatostatin (SST) has a neuroprotective action and its production is downregulated in the diabetic eye. The aim of the study was to test the hypothesis that topical administration of SST is useful in preventing retinal neurodegeneration. In addition, the effects of SST eye drops on apoptotic/survival signalling pathways and retinal glutamate levels were examined. Finally, the possibility that SST acts through upregulation of glutamate/aspartate transporter (GLAST), a major glutamate transporter in the retina which is essential for preventing glutamate accumulation was also explored.

**Material and methods:** Male Sprague Dawley rats in which diabetes was induced by streptozotocin were treated with either eye-drops of SST (n=8) or vehicle (n=8) for 15 days. Non-diabetic Sprague Dawley rats (n=8) treated with vehicle served as the control group. Electroretinography (ERG) studies were performed before starting treatment and one day prior to death. Glial activation was evaluated by measuring glial fibrillar acidic protein (GFAP) by immunofluorescence. Apoptosis was assessed by TUNEL assay. Proapoptotic molecules (FasL, caspase-8, total Bid, truncated Bid, and active caspase -3) and anti-apoptotic markers (BclxL) were measured by Western-blot. Glutamate was measured by LC-MS/MS. The expression of GLAST was assessed by RT-PCR and immunohistochemistry.

**Results:** Treatment with SST eye drops prevented both ERG abnormalities (reduction in b-wave amplitude and increase in b-wave implicit time), and the characteristic features of neurodegeneration (glial activation and apoptosis) caused by diabetes. Topical administration of SST abrogated the increase of proapoptotic signalling induced by diabetes (FasL, Bid, and activation of caspase-8 and caspase-3), and prevented the downregulation of BclxL. In addition, SST eye drops significantly reduced glutamate retinal levels and prevented the downregulation of GLAST (mRNA and protein levels) induced by diabetes.

**Conclusion:** Topical administration of SST has a potent effect in preventing the retinal neurodegenerative process that occurs in the early stages of DR. A preventive effect on the impairment of survival/apoptotic signalling induced by diabetes and a significant reduction in glutamate-induced excitotoxicity are among the mechanisms by which SST exerts its beneficial actions.

Supported by: SAF2009-07408

## 1113

### Effects of ginkgo biloba extract on prevention of development of diabetic nephropathy in type 2 diabetic patients

Y. Wang<sup>1</sup>, Z. Sun<sup>1</sup>, J. Yu<sup>2</sup>, B. Yang<sup>1</sup>, H. Yin<sup>1</sup>, Y. Wang<sup>1</sup>;

<sup>1</sup>Department of Endocrinology, Southeast University, <sup>2</sup>Department of Endocrinology, Jiangsu Province Hospital of TCM, Nanjing, China.

**Background and aims:** Diabetic nephropathy (DN) has become the leading cause of end stage renal failure, and prevention or retardation of DN has become a major goal in biomedical research. Ginkgo biloba extract (GBE) is one of the most widely used herbal medicines in the world. Our research is to observe the preventive effects of GBE on early stage of DN in type 2 diabetic patients.

**Materials and methods:** A randomized, double-blind, placebo-controlled clinical trial of 210 type 2 diabetic patients (ACR, urine albumin-to-creatinine ratio < 30mg/mmol) aged 50 to 70 years, conducted in 2 hospitals in China between 2008 and 2011. Participants received 24 mg of EGB thrice daily or placebo for 3 years. And 199 participants finished the 3-year study (GBE group, n=87; placebo group, n=112). Fasting blood glucose (FBG), postprandial blood glucose (PBG), HbA1c, total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglyceride (TG), blood creatinine (Cr), blood urea nitrogen (BUN), urine mAlb, urine Cr, ACR (urine albumin-to-creatinine ratio) were measured by different methods. Odds ratios (ORs), 95% confidence intervals were calculated using generalized estimating equations (GEE) for repeated measures, adjusting for course of disease, age, gender.

**Results:** There were no differences in FBG, PBG, TC, LDL-C, HDL-C, TG, HbA1c between GBE and placebo ( $p < 0.05$ ). To assess the effect of GBE on ACR, GEE models showed statistical significance in clinical effect between the GBE group and the placebo group ( $p = 0.02$ ). Compared with placebo, the preventive effects of GBE is better (ORs=2.17).

**Conclusion:** The use of GBE, 24 mg thrice daily, results in less ACR increase in type 2 diabetic patients in three years. GBE has the preventive effects on early stage of type 2 DN. Combined treatment of traditional Chinese medicine and western medicine may be a good treatment to the prevention or retardation of DN. Table 1. The percentage of patients that ACR < 30mg/mmol in different center in three years after GBE and placebo treatment.

Group	Center	No.	Time		
			1 year	2 year	3 year
GBE group	1	58	94.83%	87.93%	82.76%
	2	45	91.11%	97.78%	95.56%
	Total	103	92.97%	92.85%	89.16%
placebo group	1	50	82%	78%	80%
	2	46	91.3%	91.3%	89.13%
	Total	96	86.65%	84.65%	84.56%

## 1114

### Protection effect and mechanism of Compound Xue Shuantong Capsule on diabetic nephropathy

J. Yan, X. Xia, F. Xu, L. Zhang, Y. Zhao, H. Liang, J. Weng;

The third affiliated hospital of Sun Yat-Sen University, Guangzhou, China.

**Background and aims:** The overexpression of reactive oxygen species (ROS) in the presence of high glucose induces dysfunction in glomerulus mesangial cell and podocytes. Up-regulation of matrix metalloproteinase (MMP)-2 lead to metabolic disorders in the extracellular matrix (ECM). The above disorders may contribute pathologically to the development of diabetic nephropathy. Chinese Compound Xue shuantong Capsule (XST) contains four Chinese herbs: Sanqi (panax notoginseng), huangqi, danshen and scrophulariaceae. The present study was aimed to investigate protective effect and mechanism of XST on diabetic rat model with nephropathy.

**Materials and methods:** A total twenty-eight male Sprague Dawley (SD) rats were induced to hyperglycemia (three days later, fasting blood glucose > 16.7mmol/L) by peritoneal injection with streptozotocin (STZ, 50mg/kg) and divided into four groups: diabetic nephropathy group (DN, treated with vehicle), Irbesartan group (IB, 20mg/kg/d), low-dosage XST group (LX, 900mg/kg/d) and high-dosage XST group (HX, 1800mg/kg/d). Normal SD rats (n=7) were used as control group (NC). After 12-week intervention, urine protein was checked. Kidney pathological morphology was observed by HE, Masson, PAS stain and electron microscope. Blood nitric oxide (NO), superoxide dis-

mutase (SOD) and malondialdehyde (MDA) were detected. The expression of MMP-2 was detected using real time-PCR and western blotting. Analysis of variance (ANOVA) and Least-significant difference (LSD) were used for data analysis.

**Results:** Urinary albumin was decreased in three intervention group than those in DN group. Irbesartan and XST treatment had no effect on both blood glucose and bodyweight. Irbesartan and XST ameliorated pathological injury on glomerular basement membrane, podocytes, mesangial cells by HE, Masson, PAS stain and electron microscope. There were no significant difference between low-dosage XST and high-dosage XST group. The expression of MMP-2 was increased in DN group than those in NC group. Three intervention group had no effect on MMP-2 expression. The content of NO was significantly lower in IB (15.78±1.21 μmol/L), LX (41.94±9.63 μmol/L) and HX group (14.71±1.92 μmol/L) than those in DN group (104.86±11.02 μmol/L, both  $P < 0.05$ ). The content of MDA was also decreased in IB (5.12±1.64 mmol/ml), LX (6.63±0.87 mmol/ml), and HX group (4.54±1.23 mmol/ml) than those in DN group (19.60±1.56 mmol/ml, both  $P < 0.05$ ). Compared with DN group (222.22±19.78 U/ml), the activity of SOD was increased in IB (242.59±16.67 U/ml), LX (230.89±17.76 U/ml) and HX group (236.97±23.90 U/ml) (both  $P < 0.05$ ).

**Conclusion:** Compound Xue shuantong Capsule reduced urine protein and ameliorated pathological injury in kidney. The protective effect of Compound Xue shuantong Capsule may be related with suppression of oxidative stress and not with ECM metabolism.

*Supported by: National Science Fund for Distinguished Young Scholars (81025005)*

## 1115

### Dipeptidyl peptidase-4 inhibitor, vildagliptin analogue, exerts renoprotective effects independent of blood glucose lowering actions in type 1 diabetic rats

R. Koderä<sup>1</sup>, K. Shikata<sup>1,2</sup>, T. Takatsuka<sup>2</sup>, S. Miyamoto<sup>2</sup>, N. Kajitani<sup>2</sup>, D. Hirota<sup>2</sup>, D. Ogawa<sup>3</sup>, H. Makino<sup>2</sup>;

<sup>1</sup>Center for Innovative Clinical Medicine Okayama University Hospital,

<sup>2</sup>Medicine and Clinical Science, Okayama University Graduate School of Medicine Dentistry and Pharmaceutical Sciences, <sup>3</sup>Diabetic Nephropathy, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan.

**Background and aims:** We have recently shown the renoprotective effects of Glucagon like peptide-1 (GLP-1) receptor agonist, exendin-4, through anti-inflammatory effects independent of blood glucose lowering actions in type 1 diabetic rats. GLP-1 receptor is expressed on glomerular endothelial cells and monocytes/macrophages. Exendin-4 directly exerts protective effects against diabetic nephropathy by inhibition of the interaction between macrophages and glomerular endothelial cells which promotes inflammatory process. However, the renoprotective effect of dipeptidyl peptidase-4 (DPP-4) inhibitor has remained unclear. The aim of this study is to clarify the effects of DPP-4 inhibitor on diabetic nephropathy.

**Materials and methods:** Five-week old male Sprague-Dawley rats were divided into three groups; non-diabetic: ND, diabetic rats without treatment: DM, diabetic rats treated with vildagliptin analog (PKF-275): DM+PKF. Rats were administered orally with PKF-275 (10μg/kg/day, DM+PKF) or vehicle (ND and DM) every day for 8 weeks. To investigate the effects of PKF, we measured urinary albumin excretion and creatinine clearance and evaluated the renal histological morphometry. We also examined the expression of pro-inflammatory molecules in kidney tissues by quantitative real-time RT-PCR and immunostaining.

**Results:** PKF treatment significantly reduced urinary albumin excretion (Mean values; DM 699μg/day, DM+PKF 420μg/day, DM vs DM+PKF:  $P < 0.05$ ) without change of blood glucose levels and blood pressure. PKF didn't affect hyperfiltration in diabetic rats. In the renal histological morphometry, PKF significantly attenuated mesangial matrix expansion (mesangial matrix index; DM: 9.4%, DM+PKF: 8%, DM vs DM+PKF:  $P < 0.05$ ), but not glomerular size. Treatment with PKF also significantly reduced expression of intercellular adhesion molecule-1 (DM vs DM+PKF:  $P < 0.05$ ) in the cortex and glomeruli, and prevented macrophage infiltration into kidney tissues.

**Conclusion:** Treatment with PKF ameliorated albuminuria, renal tissue injuries and the expression of proinflammatory molecules in the kidney, independent of blood glucose levels and blood pressure in type 1 diabetic rats. These results suggest that PKF ameliorated diabetic renal injuries through anti-inflammatory effects. DPP-4 inhibitor as well as GLP-1 receptor agonist



might be effective for diabetic nephropathy independent of blood glucose lowering effect.

## 1116

### Low protein diet inhibits uric acid synthesis and attenuates renal damage in streptozotocin- induced diabetic rats

J. Ran<sup>1</sup>, J. Ma<sup>1</sup>, Y. Liu<sup>2</sup>, R. Tan<sup>3</sup>, H. Liu<sup>1</sup>, G. Lao<sup>1</sup>;

<sup>1</sup>Endocrinology Department, <sup>2</sup>Nephrology Department, <sup>3</sup>Institute of clinical nutrition, Guangzhou Red Cross Hospital, China.

**Background and aims:** Several studies indicated that hyperuricemia may link to the worsening of diabetic nephropathy (DN). Meanwhile, low protein diet (LPD) retards exacerbations of renal damage in chronic kidney disease. Whether LPD exert the same effect on DN through lowering blood uric acid still remains unclear. In this study, we then explore effects of LPD on uric acid metabolism and progression of renal damage in streptozotocin (STZ)-induced diabetic rats.

**Materials and methods:** Thirty STZ-induced rats were fed with LPD (8%, n=15) and normal protein diet (NPD, 18%, n=15) for 12 weeks respectively, twenty control rats were also dispatched into LPD (n=10) and NPD (n=10) group. Serum creatinine (SCr), uric acid (SUA), glucose (BG), urea nitrogen (BUN) and 24h-urine creatinine (UCr), urea nitrogen (UUN), uric acid (UUA), albumin excretion (UAE) were measured at baseline and 3, 6, 9, 12 weeks. Hepatic expressions of 3 key enzymes for uric acid synthesis including phosphoribosyl-pyrophosphat (PRPP) synthetase-1 (PRPPS-1), PRPP amidotransferase (PPAT) and xanthine oxidase (XO) were detected by RT-PCR. Finally we analysed renal histologic alterations and inflammatory factors (TNF- $\alpha$  and VEGF) expression in renal tissue.

**Results:** 1) Diabetic rats developed into constant and high level of BG, SCr, BUN, SUA as well as 24h amounts of UCr, UUN, UUA and UAE ( $P<0.01$  from the 3rd week). 2) LPD in diabetic rats significantly decreased SUA, BG and UAE ( $P<0.01$  from the 3rd week) compared with those fed with NPD, left SCr, BUN, UCr, UUN nearly compared between 2 diet groups ( $P>0.05$  from baseline). A stepwise linear regression in which UAE served as independent variable among diabetic rats at 9th and 12th week ( $B=5.82$  and  $4.40$  respectively, both  $P<0.05$ ) showed that UUA is an independent risk factor for DN progression beside BG. 3) LPD slightly inhibit PRPPS-1 ( $5.86\pm3.32$  vs.  $6.99\pm2.14$ ), PPAT ( $9.49\pm4.41$  vs.  $13.36\pm5.67$ ) and XO ( $9.24\pm7.23$  vs.  $12.15\pm6.06$ ) expression at hepatocytes. 4) Glomeruli from diabetic rats were much larger in size than control rats ( $P<0.05$ ), mesangial cell proliferation and hyaline degeneration of tubular epithelial cell were also severe ( $P<0.05$ ), but LPD remarkably ameliorated degrees of these pathologic changes ( $P<0.05$ ). 5) Expression of TNF- $\alpha$  in tubular epithelial cell significantly decreased in diabetic rats fed with LPD ( $P<0.05$ ), while VEGF expression was comparable between all rats ( $P>0.05$ ).

**Conclusion:** LPD in STZ-induced diabetic rats significantly inhibits endogenous uric acid synthesis. This process may attenuate renal damage in these rats.

Supported by: Guangzhou Scientific Fund (2010J-E251)

## PS 096 Experimental: nephropathy and retinopathy

### 1117

#### Characterisation of the morphological and functional features of the retinal neurodegenerative process in a murine model of spontaneous type 2 diabetes

P. Bogdanov<sup>1</sup>, A. Rodrigues-Carvalho<sup>2</sup>, L. Corraliza<sup>1</sup>, J. Garcia-Arumí<sup>2</sup>, J.A. Villena<sup>1</sup>, C. Hernández<sup>1</sup>, R. Simó<sup>1</sup>;

<sup>1</sup>CIBERDEM and Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute, <sup>2</sup>Ophthalmology Department, Vall d'Hebron Research Institute, Barcelona, Spain.

**Background and aims:** There is emerging evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy (DR), which antedates and participates in the microcirculatory abnormalities that occur in DR. The experimental model currently used to study retinal neurodegeneration in DR is the rat with streptozotocin-induced diabetes (STZ-DM). However, it should be noted that the interpretation of the results in this model might be hampered by the neurotoxic effect of STZ. Therefore, the aim of the present study was to characterize the sequential events that are taking place in retinal neurodegeneration in a murine model that carries a mutation in the leptin receptor gene and develops spontaneous type 2 diabetes (db/db mice).

**Material and methods:** A total of 72 C57BL/6 mice were divided into two groups: non-diabetic (db<sup>+</sup>/db<sup>+</sup>) and diabetic (db<sup>+</sup>/db<sup>+</sup>). To assess the chronological sequence of the abnormalities the analysis was performed at different evolutionary stages (8, 16 and 24 weeks). The retinas were evaluated in terms of morphological (total retinal thickness, thickness of retinal layers and ganglion cell count in HE sections) and functional abnormalities [electroretinography (ERG)]. In addition, histological markers of neurodegeneration (glial activation and apoptosis) were evaluated. Glial activation was assessed by immunohistochemistry using specific antibodies against GFAP (Glial fibrillar acidic protein). Apoptosis was quantified using the TUNEL method. Furthermore, apoptosis inducing factor (AIF) was determined by immunohistochemistry.

**Results:** At week 8 glial activation was significantly higher in diabetic than in non diabetic mice ( $p<0.01$ ). In addition, a loss of 20% ( $p<0.05$ ) of ganglion cells was detected in diabetic mice. Moreover, a significant reduction of neuroretinal thickness was also observed. All these findings were more pronounced at weeks 16 and 24. ERG abnormalities were already present at week 8 in diabetic mice and worsened in parallel with histological hallmarks of neurodegeneration. Overall all these findings are very similar than those found in the early stages of DR in diabetic patients.

**Conclusion:** Our results suggest that db/db mice are an appropriate murine model for investigating the underlying mechanisms of diabetes-induced retinal neurodegeneration and for testing neuroprotective drugs.

Supported by: EFSD/Sanofi grant and SAF2009-07408

### 1118

#### IRS2 deficiency limits the effects of inhibition of protein tyrosine phosphatase 1B in IGF-IR-mediated signalling in the retina

A.I. Arroba<sup>1,2</sup>, J. Revuelta-Cervantes<sup>1,2</sup>, L. Meses<sup>3,4</sup>, Á. González-Rodríguez<sup>1,2</sup>, P. de la Villa<sup>5</sup>, D.J. Burks<sup>3,4</sup>, Á.M. Valverde<sup>1,2</sup>;

<sup>1</sup>Centro de Investigaciones Biomédicas, Madrid, <sup>2</sup>CIBERDEM, Madrid,

<sup>3</sup>Molecular and Endocrinology Laboratory, Centro de Investigación

“Príncipe Felipe”, Valencia, <sup>4</sup>CIBERDEM, Valencia, <sup>5</sup>Universidad de Alcalá de Henares, Madrid, Spain.

**Background and aims:** Mice with complete deletion of insulin receptor substrate (IRS) 2 develop type 2 diabetes. In the retina, IRS2 deficiency induced photoreceptor degeneration and apoptosis that is not rescued by normalization of glucose levels. On the other hand, protein tyrosine phosphatase 1B (PTP1B) inhibition by genetic deletion in IRS2<sup>-/-</sup> mice (double mutant IRS2<sup>-/-</sup>PTP1B<sup>-/-</sup>) restored peripheral insulin resistance and normalized glucose homeostasis. Since IGF-IR promotes survival of photoreceptors and also is a substrate of PTP1B, we aimed to investigate IGF-IR-mediated survival signalling and visual function in PTP1B<sup>-/-</sup> and IRS2<sup>-/-</sup>/PTP1B<sup>-/-</sup> in comparison to wild-type and IRS2<sup>-/-</sup> mice.

**Materials and methods:** IGF-IR tyrosine phosphorylation and Akt serine 473 phosphorylation were analyzed by western blot in organotypic retinal

explants stimulated with IGF-I. Immunohistochemistry was used to evaluate retinal structure preservation in mice at 10–12 weeks and programmed cell death was assessed by TUNEL. Visual function was evaluated by Electroretinographic (ERG) recording in mice at 5 and 10 weeks.

**Results:** IGF-IR tyrosine phosphorylation and Akt serine 473 phosphorylation increased in retinal explants stimulated for 15 min with IGF-I in a dose-dependent manner. In PTP1B<sup>-/-</sup> retinal explants, these responses increased by twofold in IGF-IR phosphorylation and by threefold in Akt phosphorylation compared to the wild-type control ( $p=0.037$  and  $p=0.04$ , respectively). Conversely, in IRS2<sup>-/-</sup> mice the response to IGF-I in Akt phosphorylation decreased compared to the wild-type control ( $p=0.04$ ). Moreover, in IRS2<sup>-/-</sup> mice PTP1B deletion (double mutant IRS2<sup>-/-</sup>/PTP1B<sup>-/-</sup>) also enhanced IGF-IR tyrosine phosphorylation ( $p=0.01$ ) but, unexpectedly, the response to IGF-I in the activation of Akt remained decreased as observed in the IRS2<sup>-/-</sup> mice. Histological evaluation revealed a significant thickness in whole retina in both IRS2<sup>-/-</sup> and double mutant IRS2<sup>-/-</sup>/PTP1B<sup>-/-</sup> mice, specifically in the outer nuclear layer (ONL) and retinal outer segments (ROS) ( $p<0.001$ ). ERG analysis showed that PTP1B deficiency did not restore normal visual function in IRS2<sup>-/-</sup> mice.

**Conclusion:** Although PTP1B deficiency significantly enhanced IGF-IR tyrosine phosphorylation in retinal explants of IRS2<sup>-/-</sup> mice, it was unable to restore Akt phosphorylation and, therefore, the structural and functional visual of these mice defects were not improved.

*Supported by: SAF-2009 and NEURORED-DIAB*

## 1119

### Inhibition of hypoxia-induced retinal angiogenesis in nucleoside diphosphate kinase B knockout mice

Y. Feng<sup>1</sup>, V. Butenschoen<sup>1</sup>, Y. Qiu<sup>1</sup>, H.-P. Hammes<sup>2</sup>, E. Skolnik<sup>3</sup>, T. Wieland<sup>1</sup>;

<sup>1</sup>Institute of experimental and clinical pharmacology and toxicology, Mannheim, Germany, <sup>2</sup>V. Medical Clinic, Mannheim, Germany, <sup>3</sup>Division of Nephrology, New York University Langone Medical Center, USA.

**Background and aim:** Proliferative diabetic retinopathy is characterized by inappropriate angiogenesis under hypoxia which forms neovascularizations into the vitreous arising from pre-existing retinal vessels. Nucleoside diphosphate kinase B (NDPK B) can activate heterotrimeric G proteins independently of GPCRs and thus likely contributes to a variety of physiological and pathological processes. In this study we investigated the contribution of NDPK B in hypoxia-induced retinal angiogenesis.

**Materials and methods:** Retinas of NDPK B knockout mice were assessed under conditions of physiological and pathological angiogenesis. Retinal vascular morphology under physiological condition was evaluated in the NDPK B Ko mice at postnatal day 5 (p5) and p10. To study pathological angiogenesis the NDPK B Ko mice were subjected to the model of hypoxia-induced retinopathy (OIR), i.e. new born pups were kept from p7 in an oxygen incubator with 75% O<sub>2</sub> for 5 days and then transferred to room air for another 5 days until p17. Pre-retinal angiogenesis at p17 was assessed in paraffin sections stained with PAS, whereas intra-retinal vascular angiogenesis was evaluated in whole mount retina stained with vascular markers. Expression of angiogenic growth factors in the retina was measured by quantitative PCR.

**Results:** Physiological angiogenesis in NDPK B Ko mice at p5 in the retinal superficial layer as well as at p10 in the deep capillary layer did not differ from their wild type littermates. However, deficiency of NDPK B in the mice resulted in marked reduced pre-retinal angiogenesis in the OIR model compared to wild type mice ( $p<0.05$ ). Nevertheless, there was no difference in the intra-retinal angiogenesis between NDPK B Ko and wild type mice in this model. Retinal expression of VEGF, Angiopoietin-2 and Erythropoietin was highly up-regulated in the mice subjected to the OIR model compared to the mice kept in room air from p7 to p17. However, the retinal up-regulation of these markers in the OIR model was comparable in NDPK B Ko mice and their wild type littermates.

**Conclusion:** Our data indicate that NDPK B plays an essential role in pathological but not in physiological angiogenesis. The mechanism how NDPK B regulates the VEGF dependent pathological angiogenesis requires further investigation.

*Supported by: EFSD/Sanofi grant*

## 1120

### Upregulation of rab20 expression by high glucose reduces gap junction activity and accelerated death of retinal endothelial cells

C. Stottrup, S. Roy;  
Boston University, Boston, USA.

**Background and aims:** Retinal vascular cell loss is a prominent lesion associated with diabetic retinopathy, a leading cause of vision loss in the working age population. Studies have shown that gap junction intercellular communication (GJIC) through connexin 43 (Cx43) channels is essential for cell survival and maintenance of retinal homeostasis. In this study, we examined whether high glucose compromises GJIC and induces apoptosis of retinal endothelial cells through altered expression of rab20, a potential regulator of Cx43 trafficking.

**Materials and methods:** To determine whether high glucose alters rab20 protein expression, rat retinal endothelial cells (RRECs) were grown in normal (N, 5mM) or high glucose (HG, 30 mM) medium for 7 days, and total protein isolated from these cells was analyzed for rab20 protein expression using immunoprecipitation and Western blot analysis. Increasing concentrations of rab20 siRNA were used to modulate HG-induced rab20 overexpression and determine its effects on Cx43 levels and GJIC activity. Localization and distribution of Cx43 was determined by immunostaining, GJIC activity was assessed by scrape-load dye transfer technique, and cells undergoing apoptosis were identified using TUNEL assay.

**Results:** Western blot analysis revealed that HG significantly upregulated rab20 by 30% ( $P<0.01$ ) compared to cells grown in N medium. Cells grown in HG medium and transfected with either 40 nM or 80 nM rab20 siRNA showed 43% and 82% reduction in rab20 expression, respectively, compared to rab20 expression in cells grown in HG. The relative number of Cx43 plaques at sites of contact between adjacent cells was significantly reduced in HG condition by 38% ( $P<0.05$ ) compared to cells grown in N medium. Cells grown in HG medium and transfected with 40 nM or 80 nM rab20 siRNA showed increased number of Cx43 plaques by 16% and 29%, respectively ( $P<0.05$ ) and improved GJIC activity (increased number of dye-coupled cells: 21% and 68% respectively), as compared to those grown in HG medium only. Importantly, cells grown in HG medium and transfected with rab20 siRNA showed a beneficial effect in reducing the number of apoptotic cells ( $1.5 \pm 0.6$  vs.  $7.3 \pm 1.4$ ;  $P<0.01$ ).

**Conclusion:** High glucose reduces Cx43 expression and GJIC activity in part by upregulating rab20 protein expression. siRNA mediated downregulation of rab20 may improve cell-cell communication and vascular homeostasis, and prevent HG-induced vascular cell death associated with diabetic retinopathy.

*Supported by: NIH, NEI EY018218*

## 1121

### Endoplasmic reticulum stress leads to multiple manifestations of diabetic nephropathy and glomerular collagen deposition caused by high-fat diet

H. Shevalye, S. Lupachyk, P. Watcho, R. Stavniichuk, I.G. Obrosova;  
Mechanisms of Diabetes Complications, Pennington Biomedical Research Center, Baton Rouge, USA.

**Background and aims:** The endoplasmic reticulum (ER) plays a pivotal role in the folding and processing of newly synthesized proteins. Damage to ER and resultant ER stress lead to aberrant transcriptional regulation and gene expression, signaling, ion channel function, and metabolism. ER stress plays an important role in insulin resistance and obesity. We evaluated the role for ER stress in diabetic nephropathy (DN) and kidney disease caused by high-fat diet.

**Materials and methods:** In experiment 1, control and STZ-diabetic rats were treated with or without the chemical chaperone trimethylamine-N-oxide (TMAO), 110 mg kg<sup>-1</sup>d<sup>-1</sup>, for 12 wks (a prevention paradigm). Endpoints included 24-h urinary albumin (ELISA), 8-isoprostane (ELISA) and creatinine (colorimetry) excretion, kidney weight, glomerular podocyte counts (immunohistochemistry), mesangial volume and collagen deposition (histochemistry), renal cortex fibronectin, total and phospho-PERK, total and phospho-eIF2 $\alpha$ , BiP/GRP78, and CHOP (Western blot analysis), and protein carbonyl (ELISA) contents. In study 2, C57BL6/J mice were fed normal or high-fat (HFD) diets for 16 wks, and then maintained with or without a 4-wk treatment with salubrinal (1 mgkg<sup>-1</sup>d<sup>-1</sup> i.p.), a selective inhibitor of eIF2 $\alpha$  dephosphorylation. Endpoints included 24-h urinary albumin and 8-isoprostane excretion (ELISA), collagen deposition (histochemistry), and glomerular podocyte counts (immunohistochemistry).

**Results:** In experiment 1, diabetes-induced renal ER stress was manifested by increased phospho-PERK and phospho-PERK/total PERK ratio. Phospho-eIF2 $\alpha$ /total eIF2 $\alpha$  ratios and BiP/GRP78 levels were indistinguishable between control and diabetic rats. The diabetic condition was associated with 18% increase in CHOP level, but the difference with controls did not achieve statistical significance ( $p=0.073$ ). TMAO treatment reduced diabetes-associated albuminuria, mesangial expansion, fibronectin accumulation, and collagen deposition, in the absence of any effects on blood glucose concentrations, podocyte loss, urinary 8-isoprostane excretion, or renal cortex protein carbonyl accumulation. In experiment 2, salubrinal improved glucose tolerance and blunted glomerular collagen deposition in HFD-fed mice, without affecting albuminuria, glomerular podocyte loss, or urinary 8-isoprostane excretion.

**Conclusion:** ER stress is implicated in the development of multiple manifestations of DN and glomerular collagen deposition caused by HFD consumption. These results provide the rationale for further studies of ER stress/unfolded protein response in prediabetic and diabetic kidney disease, and identify a new therapeutic direction.

## 1122

### Rho/ROCK and p38 MAP kinase signalling pathways drive the effect of fluctuating glucose on renal fibrogenesis

S. Hadjadj<sup>1</sup>, T. Harnois<sup>2</sup>, S. Brishoual<sup>3</sup>, I. Paris<sup>4</sup>, E. Vincent-Tassin<sup>2</sup>, P.-J. Saulnier<sup>5</sup>, N. Bourmeyster<sup>6</sup>;

<sup>1</sup>Endocrinology -Diabetology, CHU Poitiers, <sup>2</sup>Endocrinology -Diabetology, CHU Poitiers, <sup>3</sup>CIC 0802, CHU Poitiers, <sup>4</sup>EA 4331, Université de Poitiers, <sup>5</sup>INSERM CIC 802, CHU Poitiers, <sup>6</sup>Institut de Physiologie et Biologie cellulaires, CHU Poitiers, France.

**Background:** Glucose fluctuations recently emerged as a possible mechanism promoting vascular complications in addition to glucose exposure. How glucose fluctuations promote renal fibrogenesis is still ill-defined.

**Materials and methods:** We used two cell models to answer this question: a cell-line derived from rat mesangial cells (NRK-49) and a primary culture of skin fibroblasts from healthy volunteers. We changed the milieu every two hours for ten hours, for two consecutive days to compare the effect of glucose concentration in the medium: normal glucose - NG - (5.6 mM), high glucose-HG- (25 mM), fluctuating glucose -FG- (alternance of 5.6 and 25 mM).

**Results:** Fluctuating glucose (FG) promoted renal fibrogenesis (Fibronectin protein expression) in both cell models compared to non-fluctuating normal and even high glucose. This effect was not related to activation of the ERK, JNK or NF $\kappa$ B pathway, while phospho-p38 MAP-kinase was significantly increased in FG conditions compared to NG and also to HG. There was a specific activation of Rho/ROCK by FG with RhoA and MyPT-1 activation. Adding P38 MAP kinase and Rho kinase inhibitor Y27632 resulted in a normalisation of fibronectin secretion with an additional effect of both pathways to reverse this phenotype.

**Conclusion:** We conclude that the effect of fluctuating glucose to promote renal fibrogenesis was evidenced in two independent models of mesangial cells and was related to p38 MAP-kinase and Rho signaling, leading to a peculiar interest of this pathway for diabetic nephropathy. The identification of the Rho/ROCK pathway as a specific activated pathway following glucose fluctuations might lead to a better understanding of diabetes complications pathophysiology.

Supported by: Ministère de la santé PHRC 2004

## 1123

### Histamine H<sub>4</sub> receptors are present in the kidney of diabetic rats

A.C. Rosa<sup>1</sup>, C. Grange<sup>2</sup>, A. Pini<sup>3</sup>, M.A. Katebe<sup>4</sup>, E. Benetti<sup>1</sup>, M. Collino<sup>1</sup>, G. Miglio<sup>1</sup>, D. Bani<sup>5</sup>, G. Camussi<sup>2</sup>, P.L. Chazot<sup>4</sup>, R. Fantozzi<sup>1</sup>;

<sup>1</sup>Scienza e Tecnologia del Farmaco, University of Turin, Italy, <sup>2</sup>Department of Internal Medicine, Centre for Molecular Biotechnology, University of Turin, Italy, <sup>3</sup>Department of Preclinical and Clinical Pharmacology, University of Florence, Italy, <sup>4</sup>School of Biological and Biomedical Sciences and Wolfson Institute, Durham University, UK, <sup>5</sup>Department of Anatomy, Histology and Forensic Medicine, Section of Histology, University of Florence, Italy.

**Background and aims:** Histamine, a biogenic amine that exerts its effects through the interaction with four subtypes of G-protein-coupled receptors designated H<sub>1-4</sub>, was previously suggested to be involved in diabetic-related kidney disease. While the renal expression of H<sub>1</sub> and H<sub>2</sub> receptors was

demonstrated, that of the most recently discovered H<sub>3</sub> and H<sub>4</sub> receptors was poorly investigated. The aim of this research was to investigate the expression of the H<sub>4</sub> receptors in the kidney of healthy and diabetic rats.

**Materials and methods:** Diabetes was induced in 12 out of 24 8-week-old male Wistar rats by a single i.v. injection of streptozotocin, and animals were sacrificed 6 weeks later. Kidneys were collected and processed for RT-PCR or immunohistochemistry analyses. To ascertain the renal distribution of H<sub>4</sub> receptor, colocalization experiments were performed by using tubular markers, such as aquaporin 1, 2 and 3, Na-K-Cl cotransporter 1, Tissue non-specific alkaline phosphatase and Tamm-Horsfall glycoprotein.

**Results:** Diabetic rats showed a kidney-to-body weight ratio significantly higher and a creatinine clearance decreased. The increase in urinary N-Acetyl- $\beta$ -glucosaminidase level suggested tubular dysfunction. These biochemical abnormalities were associated with clear changes in renal histology. H<sub>4</sub> receptor was expressed in the kidney of healthy rats, although at a very low level, and was profoundly upregulated in diabetic animals. Immunohistochemical detection demonstrated that H<sub>4</sub> receptor subtype was not present in the glomeruli but in the tubules, with the highest immune-positivity in the medulla. Comparing H<sub>4</sub> receptor immunostaining to that of tubular markers a close overlap in expression topology was observed only with Tamm-Horsfall glycoprotein, thus indicating that the receptor is expressed on epithelial cells of the thick ascending limb of the loop of Henlé.

**Conclusions:** The results demonstrate for the first time that H<sub>4</sub> receptor is expressed by resident renal cells of the thick ascending limb of the loop of Henlé and that the receptor is significantly overexpressed in diabetic animals, thus suggesting a possible role of this receptor in the (physio) pathogenesis of diabetic-associated renal disease.

Supported by: Royal College of Anaesthesia/BJA, COST Action BM0806, University of Turin

## 1124

### Investigation of the podocyte-secreted protein R3h domain containing-like in diabetic nephropathy

I. Takahiro<sup>1</sup>, M. Takemoto<sup>1</sup>, Y. Akimoto<sup>2</sup>, K. Yan<sup>3</sup>, S. Onishi<sup>1</sup>, E. Okabe<sup>1</sup>, P. He<sup>1</sup>, R. Ishibashi<sup>1</sup>, K. Kobayashi<sup>1</sup>, M. Fujimoto<sup>1</sup>, H. Kawamura<sup>1</sup>, C. Betsholtz<sup>4</sup>, K. Tryggvason<sup>4</sup>, K. Yokote<sup>1</sup>;

<sup>1</sup>Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Japan, <sup>2</sup>Division of Microscopic Anatomy, Department of Anatomy, Kyorin University School of Medicine, Tokyo, Japan, <sup>3</sup>Department of Pediatrics, Kyorin University School of Medicine, Tokyo, Japan, <sup>4</sup>Department of Medical Biochemistry and Biophysics, Division of Matrix Biology, Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** Diabetic nephropathy (DN) is by far the most common cause of end-stage renal disease. However, the molecular mechanisms of DN are largely unknown. Recent discoveries of podocyte-expressed genes have considerably enhanced our knowledge of the molecular mechanism of glomerular filtration by the kidney. We previously reported the identification of a podocyte-specific transcript, R3H domain containing-like (R3hdm1), as a novel regulator of the glomerular basement membrane in EASD 2011. The aim of this study was to further analyze the functions of R3hdm1 in vivo and in vitro, particularly in DN.

**Materials and methods:** R3hdm1 mRNA was stably expressed in human embryonic kidney cells (Ad293), and protein expression was evaluated by immunohistochemistry (IHC) or western blotting (WB). R3hdm1 protein was purified using an anti-R3hdm1 antibody-conjugated column. Purified R3hdm1 protein (p-R3hdm1) was added to the cultured podocytes and endothelial cells, and the localization of R3hdm1 protein was evaluated by IHC and WB. The effects of transforming growth factor- $\beta$  (TGF- $\beta$ ) on R3hdm1 expression and function were evaluated by RT-PCR. Expression of urinary R3hdm1 protein in diabetic mice (Akita mice) was compared with that in wild-type mice.

**Results:** IHC revealed that R3hdm1 protein was mainly localized to the Golgi apparatus in the cytoplasm. WB analyses detected two bands (approximately 33 and 35 kDa) in the cytoplasm. WB analyses also revealed R3hdm1 protein as a 20-kDa protein in the conditioned medium from R3hdm1-overexpressing Ad293 cells but not in the medium from control cells. These results indicated that R3hdm1 proteins were posttranscriptionally modified, processed, and secreted from the cells. When we added p-R3hdm1 to the cultured podocytes and endothelial cells, R3hdm1 protein mainly localized to the nuclei of the cells, without increasing R3hdm1 mRNA expression. TGF- $\beta$  has been reported to play major roles in DN. Therefore, we evaluated the effects of TGF- $\beta$  on R3hdm1 expression and function. TGF- $\beta$  increased the expression



of R3hdml in cultured podocytes at the mRNA and protein levels and that of fibronectin mRNA in podocytes. In the absence of R3hdml, TGF- $\beta$  increased the expression of fibronectin even further. These results suggested that the expression of R3hdml was regulated by TGF- $\beta$  and R3hdml negatively regulated TGF- $\beta$  signaling. Finally, we investigated the expression of R3hdml proteins in diabetic animals and found that its expression was significantly increased in diabetic animals compared with that in wild-type controls.

**Conclusion:** We identified R3hdml as a novel podocyte-specific gene. R3hdml protein is secreted from podocytes, and its expression is increased in DN. R3hdml might negatively regulate TGF- $\beta$  signaling. Therefore, R3hdml may be useful as a novel DN biomarker and/or a new drug target for DN.

## 1125

### IRS 2 is an essential molecule for insulin signalling in renal cells

B. Santamaría<sup>1,2</sup>, A. Gonzalez-Rodriguez<sup>1,2</sup>, G.I. Welsh<sup>3</sup>, L. Ni<sup>3</sup>, L. Hale<sup>3</sup>, M.A. Saleem<sup>3</sup>, R.J. Coward<sup>3</sup>, A.M. Valverde<sup>1,2</sup>;

<sup>1</sup>Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-UAM,

<sup>2</sup>CIBERDEM, Madrid, Spain, <sup>3</sup>Academic Renal Unit, School of Clinical Sciences, University of Bristol, UK.

**Background and aims:** Diabetic nephropathy is the commonest cause of end-stage renal failure in the world. Its natural history is dominated by progressive albuminuria. Recently it has been demonstrated that podocyte insulin resistance may be implicated in this diabetic complication. Podocytes are crucial in maintaining integrity of the glomerular filtration barrier and preventing albuminuria. On this basis, we investigated the role of the IRS 2 in the development of diabetic nephropathy and specifically if IRS 2 affects insulin signalling in kidney function.

**Materials and methods:** We have used an *in vivo* and *in vitro* approach to study the role of IRS2 in the podocyte of the glomerulus. Initially we studied the renal phenotype of whole body IRS2 deficient mice. We then generated conditionally immortalised murine podocyte cell lines from these mice using a temperature sensitive SV40 large T antigen construct to study its role in insulin signalling in this cell.

**Results:** IRS2-deficient (IRS2<sup>-/-</sup>) animals present defects in both insulin action and insulin production. By 12 weeks of age, a proportion of IRS2<sup>-/-</sup> mice are diabetic and, therefore, they exhibit a severe hyperglycemia (glucose levels >400 mg/dl) and develop significant levels of albuminuria. They exhibit mild glomerular abnormalities (matrix accumulation) using light microscopy and have podocyte abnormalities using electron microscopy with significant foot process widening. Fascinatingly the immortalised cells from these mice are profoundly insulin resistant in comparison to podocytes derived from age-matched wild-type mouse controls (insulin-stimulated increase in glucose uptake 150 % WT vs 100 % IRS2 KO). They also have significantly impaired AKT (45 % PKB) signalling in response to insulin and exhibit impaired GLUT4 membrane translocation. They have comparable levels of IRS1 in comparison to controls.

**Conclusion:** These results show suggest the critical importance of IRS2 in maintaining glomerular function in glomeruli being critical for normal kidney function. Furthermore, the *in vitro* studies demonstrate that IRS2 is a critical node for insulin signalling in the podocytes.

Supported by: SAF2009-08114 (2010-2012), Spanish Ministry of Science and Technology

## 1126

### Endothelial function and myogenic constriction of small renal arteries as determinants of diabetic kidney damage in Zucker diabetic fatty rats

H. Buikema, M. Hamidi Shishavan, S. Landheer, L.E. Deelman,

R.H. Henning;

Clinical Pharmacology, University Medical Center Groningen / University of Groningen, Netherlands.

**Background and aims:** Endothelial dilatory function (EDF) and smooth muscle myogenic constriction (MC) are important mechanisms in autoregulation of renal blood flow and glomerular filtration rate (GFR), but also in the protection of renal structures from systemic blood pressure. Consistent with that, we found interindividual variability in intra-renal EDF and MC to predict and precede kidney damage. Here we studied intra-renal EDF and MC as predictors of renal damage development in obese Zucker Diabetic Fatty rats (ZDF).

**Materials and methods:** ZDF (n=14) underwent unilateral nephrectomy (UNx) at 7 weeks of age. Small arteries were isolated from the extirpated kid-

ney and studied *in vitro* for EDF to acetylcholine and pressure-induced MC (in endothelium-denuded segments). Baseline EDF- and MC-values were ranked from low-to-high for individual rats (1-14), and an EDF\*MC interaction ranking was calculated additionally. Rats were followed until 10 weeks thereafter when final metabolic and renal function parameters were assessed and blood pressure determined. Baseline renal vascular function (EDF and MC solitary ranking, and EDF\*MC interaction ranking) was analysed to predict the development of proteinuria following UNx by means of correlation analysis.

**Results:** Metabolic and renal parameters confirmed that ZDF were at pre-diabetic state with intact renal function when baseline renal vascular function was assessed. Ten weeks later, blood glucose had increased to 16.8±2.3 mmol/L, HbA1c to 5.6±0.3%, and 24h urinary protein excretion to 188.4±20.5 mg, while systolic blood pressure (120±3 mmHg) was still within normotensive range. Correlation analysis showed that EDF\*MC interaction ranking was positively correlated with baseline GFR ( $r^2=0.47$   $p=0.04$ ), suggesting it to be an *in vitro* derivative of *in vivo* renal vascular function. Furthermore, baseline EDF\*MC interaction ranking (but not EDF and MC solitary ranking) inversely correlated with the degree of proteinuria development following UNx in ZDF rat ( $r^2=-0.47$ ,  $p=0.03$ ).

**Conclusion:** The findings demonstrate that unlike in models of hypertension related kidney disease, EDF and MC as solitary components of renal vascular function did not predict the individual's susceptibility to renal damage in diabetic conditions in the absence of high blood pressure. However, the interaction between the both - which we calculated as the EDF\*MC interaction ranking - significantly correlated to proteinuria development as an indices of renal damage following UNx in individual ZDF rat. EDF\*MC interaction ranking may represent a new an *in vitro* derivative of *in vivo* renal vascular function with predictive-value for susceptibility to renal damage development in non-hypertensive conditions.

## PS 097 Predicting nephropathy and retinopathy

1127

### Association of monocyte chemoattractant protein-1 (MCP-1) 2518 A/G polymorphism with a proliferative diabetic retinopathy (PDR) in Korean type 2 diabetes

T. Oh<sup>1</sup>, H.-J. Jeon<sup>1</sup>, Y.-H. Lee<sup>2</sup>;

<sup>1</sup>Internal Medicine, <sup>2</sup>Biochemistry, Chungbuk National University, Cheongju, Republic of Korea.

**Background and aims:** Monocyte chemoattractant protein-1(MCP-1) is a chemokine that exert several effects on monocytes, including the production of superoxide anion, cytokine production, and adhesion molecule expression. It has been reported that hyperglycemia increases MCP-1 production in vascular endothelial cells and retinal pigmented epithelial cells. In this study, we evaluated the association of the MCP-1 2518 A/G polymorphism with proliferative diabetic retinopathy (PDR) in Korean type 2 diabetes.

**Materials and methods:** We conducted a case-control study, which enrolled 590 patients with Korean type 2 diabetes. Diabetic retinopathy was defined by the presence of characteristic changes, including hemorrhages, exudates or history of laser photocoagulation or vitrectomy. Genotyping of MCP-1 2518 A/G polymorphism was performed using polymerase chain reaction followed by digestion with Pvu II restriction enzyme.

**Results:** The prevalence of the MCP-1 2518 A/G genotype was as follows: AA, 13.2%; AG, 47.1%; GG, 39.7%; and the genotype distribution was in Hardy-Weinberg equilibrium. In terms of severity of diabetic retinopathy, the prevalence of proliferative diabetic retinopathy (PDR) was significantly higher in subjects with AA genotype compared with those with AG or GG genotypes [35.9%(28/78) vs.22.3%(114/512),  $P=0.009$ ]. The prevalence of any other diabetic micro or macro complications such as neuropathy, nephropathy, and cardiovascular events was not different according to the MCP-1 2518 A/G genotype.

**Conclusion:** Our results suggest that the AA genotype of MCP-1 2518 A/G polymorphism could be a susceptibility gene for the PDR in Korean type 2 diabetic patients.

1128

### Progression of diabetic nephropathy and genetic variability in the renin-angiotensin-aldosterone system: evidence for pharmacogenetic effects?

V. Bartakova<sup>1</sup>, D. Maluskova<sup>2</sup>, K. Kuricova<sup>1</sup>, V. Tanhauserova<sup>1</sup>, L. Pacal<sup>1</sup>, J. Belobradkova<sup>3</sup>, J. Muzik<sup>2</sup>, T. Pavlik<sup>2</sup>, J. Svojanovsky<sup>4</sup>, D. Krusova<sup>4</sup>, J. Rehorova<sup>3</sup>, K. Kankova<sup>1</sup>;

<sup>1</sup>Dept. of Pathophysiology, Masaryk University Brno, Faculty of Medicine,

<sup>2</sup>Institute of biostatistics and analyses, Masaryk University Brno, Faculty of Medicine and Faculty of Science, <sup>3</sup>Dept. of Gastroenterology, Faculty Hospital Brno-Bohunice, <sup>4</sup>Dept. of Internal Medicine, St. Anne's Faculty Hospital, Brno, Czech Republic.

**Background and aims:** Pathological over-activation of renin-angiotensin-aldosterone system (RAAS) as the key pathogenic mechanism of diabetic nephropathy (DN). Pharmacological blockade of RAAS represents main renoprotective treatment of DN. Several single nucleotide polymorphisms (SNPs) were shown to influence interindividual variability in RAAS (e.g circulating levels of RAAS compounds and enzyme activities) and could hypothetically also modify therapeutic effect of RAAS blockers. We aimed to study contribution of genetic variability in RAAS to the progression of DN and other adverse outcomes of diabetes taking into account RAAS blocking treatment in a quantitative manner.

**Materials and methods:** A total of 391 diabetic patients with variable stage of kidney disease were prospectively followed for a median of 39 (IQR 20–58) months. We considered following end-points: (1) progression of DN, (2) major cardiovascular event (MCVE, non fatal myocardial infarction or stroke) and (3) all-cause mortality. Selected SNPs were genotyped using PCR: angiotensin converting enzyme (I/D ACE), angiotensinogen (ATG M235T), angiotensin II type I receptor (ATRI A1166C), aldosterone synthase (CYP11B2-344T/C), hydroxysteroid dehydrogenase (HSD11B1 8355T/insA, HSD11B2 G534A) and mineralocorticoid receptor (MR G3514C, MR A4582C). Subjects were receiving following antihypertensive treatment: (1) angiotensin converting enzyme inhibitors only (ACEi, 36%), (2) angiotensin II receptor

blockers only (ARBs, 14%), (3) both ACEi and ARBs (23%) and (4) no antihypertensive treatment (27%). Cumulative dose during the follow-up was expressed as units of Captopril for ACEi and units of Losartan for ARBs.

**Results:** We found significant differences in mortality between carriers of genotypes of CYP11B2 -344T/C and ATG M235T (log-rank test,  $P=0.05$  and  $0.037$ , respectively) in univariate time-to-event analysis. Using Cox regression model carriers of the combination of risk genotypes exhibited significant difference in mortality (HR 2.70 95% CI 1.34-5.43,  $p=0.005$ ) without significant contribution of treatment parameters.

**Conclusion:** We identified significant genetic contribution of genetic variability in the RAAS to all-cause mortality of diabetic patients however no evidence of pharmacogenetic effects. More thorough analysis are currently under way.

Supported by: Ministry of Health of Czech Republic NT/13198

1129

### The association of the 174G>C polymorphism on interleukin 6 gene with diabetic nephropathy in patients with type 2 diabetes mellitus

S. Papaoikonomou<sup>1</sup>, N. Tentolouris<sup>1</sup>, D. Tousoulis<sup>2</sup>, D. Papadogiannis<sup>1</sup>, A. Miliou<sup>2</sup>, G. Hatzis<sup>2</sup>, N. Papageorgiou<sup>2</sup>, A. Kokkinos<sup>1</sup>, N. Katsilambros<sup>1</sup>, C. Stefanadis<sup>2</sup>;

<sup>1</sup>1st Department of Propaedeutic and Internal Medicine, University of Athens Medical School, Laiko General Hospital, <sup>2</sup>1st Cardiology Department, University of Athens Medical School, Hippokration General Hospital, Athens, Greece.

**Background and aims:** Chronic inflammatory processes are thought to play a key role in the development of microvascular complications in type 2 diabetes mellitus. C-reactive protein (CRP) is a marker of systemic inflammation and is associated with sub-clinical measures of atherosclerosis. Microalbuminuria and subsequent progression to proteinuria and nephropathy is associated with increased oxidative stress and inflammatory cytokines, but whether common polymorphisms of inflammatory genes are associated with levels of albumin excretion and progression of nephropathy are still unknown. The aim of the present study was to examine the association of 174GC polymorphism of interleukin-6 (IL6) gene with diabetic nephropathy in patients with type 2 diabetes mellitus (T2DM).

**Materials and methods:** The study population consisted of 431 patients with T2DM (mean age  $66.5 \pm 9.96$  years, male  $n=218$ , female  $n=213$ ). The IL6174GC polymorphism was detected by polymerase chain reaction and appropriate restriction enzyme digestion (SFANI). Hs-CRP was assayed by particle-enhanced immunonephelometry and 24h albumin excretion was measured by analytical methods. Diabetic nephropathy was defined as presence of microalbuminuria and/or proteinuria.

**Results:** The genotype distribution was 49.1% GG, 26.8% GC and 24.1% CC, with no significant gender difference. Hs-CRP levels (mg/l) did not differ among the three genotypes. The CC homozygotes had lower albumin excretion (mg/24h) in comparison with the GC genotype, CC:  $8.9(4.0-20.9)$  vs GC:  $21.95(9.1-53.35)$ , ( $p=0.004$ ). Participants with the GC genotype tended to have more frequently nephropathy than those with the GG and the CC genotype (presence/absence of nephropathy; GC:  $(44.55/53.6\%)$  vs GG:  $(35.1\%/60.4\%)$  and CC:  $(28.3\%/66.7\%)$  genotypes, ( $p=0.071$ ). Multivariate logistic regression analysis demonstrated that the CC homozygotes had lower odds to have nephropathy in comparison with the GC genotype (odds ratio: 0.51, 95% confidence intervals=0.28-0.91,  $p=0.024$ ). This association remained significant after adjustment for gender, age, duration of diabetes, body mass index, smoking, hypertension, lipids, HbA1c and glomerular filtration rate ( $p=0.014$ ).

**Conclusion:** The IL6174GC polymorphism affects the levels of albumin excretion and the development of nephropathy in patients with T2DM, but not the hs-CRP levels. Specifically, the CC homozygotes have lower levels of 24h albumin excretion and are protected from the presence of nephropathy in comparison with the GC genotype.

## 1130

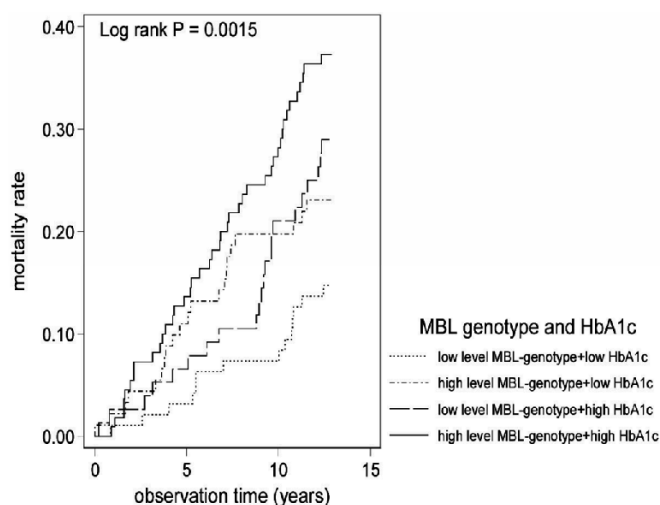
**Mannose-binding lectin (MBL) genotype associated with all-cause mortality in type 1 diabetes**J.A. Østergaard<sup>1</sup>, M.S. Lajer<sup>2</sup>, P. Rossing<sup>2</sup>, A. Flyvbjerg<sup>1</sup>, L. Tarnow<sup>2,3</sup>, T.K. Hansen<sup>1</sup><sup>1</sup>Dept. of Endocrinol. and Int. Med. and Med. Res. Labs., Aarhus University Hospital, <sup>2</sup>Steno Diabetes Center, Gentofte, Denmark, <sup>3</sup>Aarhus University, Denmark.

**Background and aims:** Mannose-binding lectin (MBL) can initiate the complement system by carbohydrate recognition. MBL levels vary many-fold between subjects due to polymorphisms. Type 1 diabetes patients have higher MBL level than healthy subjects and high level MBL-genotypes and MBL concentrations are associated with risk of micro- and macrovascular complications. However, the precise link between MBL and mortality and cardiovascular disease remains unresolved.

**Materials and methods:** We studied effects of MBL-genotype on mortality by following 371 type 1 diabetes patients for 12 years (197 with diabetic nephropathy, 174 with normoalbuminuria). MBL genotypes were divided into high level or low level MBL-genotype by promoter and exon 1 polymorphisms.

**Results:** During follow-up, 97 patients died (78 with nephropathy). All-cause mortality was significantly higher in patients with high level MBL-genotypes compared with low level MBL-genotypes, hazard ratio 1.6 (95% CI 1.1–2.5),  $P=0.018$  by Cox regression analysis including baseline  $HbA_{1c}$  as covariate. The effect of genotype was not modified by  $HbA_{1c}$ . There was a weak positive correlation between baseline serum MBL and  $HbA_{1c}$  in all patients (correlation coefficient 0.10,  $P=0.048$ ) and in patients with high level MBL-genotype (correlation coefficient 0.33,  $P<0.0001$ ). A four-step variable with high level or low level MBL-genotype in combination with  $HbA_{1c}$  above or below the median (9.0%) significantly described all-cause mortality rates (Figure 1), log rank  $P=0.0015$ . Interestingly, hazard ratios did not differ between patients with poor glycaemic control and high level MBL-genotype and patients with better glycaemic control and low level MBL-genotype. By contrast, the mortality in the high level MBL-genotype/high  $HbA_{1c}$  group was 37% during follow-up, compared to 15% in the low level MBL-genotype/low  $HbA_{1c}$  group; hazard ratio 3.2 (CI 1.7–5.9),  $P<0.001$  by unadjusted Cox regression analysis. After adjustment for age, sex, smoking, systolic blood pressure, cholesterol and diabetic nephropathy, the hazard ratio was 2.0 (1.1–3.8),  $P=0.032$ .

**Conclusion:** Our findings demonstrate a significant independent impact of MBL-genotype on all-cause mortality in the same order of magnitude as poor glycaemic control.



## 1131

**Urinary adiponectin concentration is associated with microalbuminuria in glucose intolerance patients**

W. Jeon, C. Park, S. Park, E. Rhee, W. Lee, K. Oh, S. Park;

Endocrinology and Metabolism, Kangbuk Samsung Hospital, Seoul, Republic of Korea.

**Background and aims:** Inverse relationship between serum adiponectin and insulin resistance or type 2 diabetes mellitus has been reported. This study aimed to investigate the role of urinary adiponectin on glucose homeostasis and albumin level in the urine.

**Materials and methods:** Six hundred twenty subjects (Prediabetes subjects=144, type 2 diabetes mellitus subjects=476) were participated in this study. The level of urine adiponectin was measured by enzyme linked immunosorbent assay (ELISA) kit (Adipogen, Korea). Urinary albumin excretion was assessed by ratio of urinary albumin to creatinine (ACR). Insulin resistance was determined by homeostasis model assessment index (HOMA-IR).

**Results:** Urinary adiponectin levels were significantly higher in patients with type 2 diabetes mellitus than prediabetes subjects ( $P=0.027$ ) and similar to those in subjects with microalbuminuria compared to normoalbuminuria participants ( $P<0.001$ ). Urinary adiponectin levels were positively correlated with age, fasting plasma glucose, post-prandial glucose,  $HbA_{1c}$ , triglyceride, HOMA-IR, blood pressure, hs-CRP, brachial-ankle pulse wave velocity, and the ratio of urinary albumin to creatinine (ACR) (all  $P<0.05$ ). Urinary adiponectin levels were significantly associated with risk of type 2 diabetes mellitus (OR 1.024, 95% CI 1.001–1.048,  $P=0.037$ ). Also, the adiponectin amount in urine showed significantly higher risk for microalbuminuria after adjusting age, sex and other metabolic parameters (OR 1.030, 95% CI 1.013–1.047,  $P<0.001$ ).

**Conclusion:** In conclusion, urinary adiponectin concentration is positively associated with microalbuminuria. Therefore, we suggest that measurement of urinary adiponectin level is an independent indicator of microalbuminuria.

## 1132

**Proximal and distal tubular markers in patients with type 1 diabetes**

B. von Scholten, S. Theilade, M. Lajer, C. Joergensen, S.E. Nielsen, P. Rossing; Steno Diabetes Center, Gentofte, Denmark.

**Background and aims:** The isoenzymes alpha and pi glutathion S-transferase ( $\alpha$ -GST and  $\pi$ -GST) are secreted from the proximal and distal part of the renal tubules, respectively. Levels of both enzymes were found elevated in type 2 diabetes and acute tubular damage, with further increase of  $\pi$ -GST with increasing urinary albumin excretion, whereas in another study elevated  $\alpha$ -GST indicated preserved renal function. The aim of this study was to evaluate if levels of  $\alpha$ -GST and  $\pi$ -GST were elevated and associated with albuminuria and renal function in patients with type 1 diabetes.

**Materials and methods:** Cross sectional study including 187 patients and 16 control persons. Patients were (mean $\pm$ SD) 53 $\pm$ 14 years, with 29 $\pm$ 18 years diabetes duration, 123(66%) were male. Normoalbuminuria (300mg/24-hour) in 70(37%) of patients. Controls were 40 $\pm$ 12 years old and 11(69%) were male. Morning spot urines were analysed with ELISA methods (Argutus Medical).

**Results:** Median(range)  $\pi$ -GST in controls was 9.4(1.5–77.5) ug/l, in normoalbuminuric 9.3(0.3–172.0) ug/l, in microalbuminuric 9.0(0.3–340) ug/l and in macroalbuminuric 6.1(0.3–138) ug/l ( $p=0.191$ ). In controls  $\alpha$ -GST was 9.3(0.3–50.5) ug/l, and in patients with normoalbuminuria 4.3(0.3–50.8) ug/l, with microalbuminuria 2.5(0.3–170.8) ug/l and with macroalbuminuria 3.0(0.3–18.3) ug/l ( $p=0.025$ ).  $\pi$ -GST was lower in men vs. women (7.5(0.3–90.3) vs. 15.0(0.8–340.0);  $p<0.05$ ). When comparing all patients to controls, levels of  $\alpha$ -GST were 3.0(0.3–170.8) ug/l in the patient group and 9.3(0.3–50.5) in the control group, while levels of  $\pi$ -GST were 8.8(0.3–340.0) ug/l in the patient group and 9.4(1.5–77.5) ug/l in the control group.  $\alpha$ - and  $\pi$ -GST correlated with each other ( $r=0.357$ ;  $p<0.001$ ), and  $\alpha$ -GST correlated with  $HbA_{1c}$  ( $r=0.179$ ;  $p=0.001$ ) and eGFR ( $r=0.169$ ;  $p=0.016$ ); whereas  $\pi$ -GST correlated with none of these covariates ( $p>0.05$ ). Neither GST isoenzymes correlated with age, duration of diabetes, total cholesterol, 24-hour SBP or urinary albumin excretion rate ( $p>0.05$ ).

**Conclusion:** Urinary levels of the tubular damage marker  $\pi$ -GST were similar in controls and patients with or without elevated u-albumin, whereas urinary levels of  $\alpha$ -GST were lower in diabetes and decreased further with increasing albuminuria state or impaired renal function. These findings in



chronic renal damage are in contrast to the findings in acute tubular damage with increased levels of the markers.

Clinical Trial Registration Number: NCT01171248

## 1133

### Urinary exosomal microRNA signature in incipient diabetic nephropathy

F. Barutta, M. Tricarico, A. Corbelli, S. Pinach, S. Grimaldi, G. Bruno, M. Rastaldi, P. Cavallo Perin, G. Gruden;  
Department of Internal Medicine, University of Turin, Italy.

**Background and aims:** Diabetic nephropathy (DN) is the leading cause of end-stage renal failure in the Western World. MicroRNAs (miRNAs) are a class of small (20–22 nucleotides) non-protein-encoding RNAs that regulate gene expression via suppression of target mRNAs and have a critical role in many pathophysiological processes. MiRNAs are present in body fluids in a remarkable stable form as packaged in microvesicles of endocytic origin, named exosomes. Circulating miRNAs can display unique expression profiles in pathological conditions, suggesting that distinctive miRNA signatures may be exploited as diagnostic/prognostic tools. MiRNAs are also found in the urine and, in the present study, we compared miRNA expression profile of urinary exosomes in type 1 diabetic patients (DM1) with and without incipient DN.

**Materials and methods:** Urine were collected overnight from DM1 with and without microalbuminuria (n=10 per group), comparable for age, sex, diabetes duration, and HbA<sub>1c</sub>. Urinary exosomes were isolated from pre-cleared urine by ultracentrifugation. Exosomes quality and purity was assessed by electron microscopy, immunoblotting, (presence/absence: HSP70, alix, calnexin), and spectrophotometry. Exosomal total RNA was extracted, reverse-transcribed and pre-amplified. The Human Taqman miRNA Arrays Card A (Applied Biosystem) was used to performed initial screening of 376 miRNAs expression in 2 micro- and 2 normoalbuminuric patients. Among differentially expressed miRNA with Ct values ≤ 35 (SDS software), five miRNAs were selected on the basis of “in silico” analysis and their expression measured in all samples by real-time PCR.

**Results:** Vesicles were <100 nm in size with a mode of 35–40 nm, showed a characteristic cup-shaped morphology at the electron microscopy, and expressed both the exosome markers HSP70 and Alix, while were negative for calnexin, an endoplasmic reticulum protein. Electropherograms of extracted RNA showed that exosomes were highly enriched in small RNA species, including miRNAs, whereas no ribosomal RNA was detected. MiRNA expression profiling identified 245 miRNAs expressed in urinary exosomes from DM1 patients of which 88 miRNAs were downregulated and 48 upregulated. In silico analysis revealed that 5 differently expressed miRNAs were expressed by glomerular cells (mesangial cells: miR-145; podocytes: miR-155, miR-424; endothelial cells: miR-130a miR-199a-5p). Validation of profiling results demonstrated that miR-130a, miR-199a-5p, miR-145 expression was significantly higher, while miR-155 and miR-424 significantly lower in micro- than in normoalbuminuric patients (p<0.05).

**Conclusion:** These results show that the expression of urinary exosomal miRNAs differs in DM1 with and without incipient DN and may represent an innovative diagnostic/prognostic tool.

Supported by: *Compagnia di San Paolo*

## 1134

### Activation of CD40/CD40 ligand system is associated with nephropathy in type 1 diabetic patients

V.V. Klimontov<sup>1</sup>, I.A. Bondar<sup>1</sup>, E.M. Parfentjeva<sup>1</sup>, V.V. Romanov<sup>2</sup>, A.P. Nadeev<sup>1</sup>;

<sup>1</sup>Novosibirsk State Medical University, <sup>2</sup>Laboratory INVITRO, Novosibirsk, Russian Federation.

**Background and aims:** The changes in extracellular matrix remodeling and chronic low-grade inflammation are believed to play an important role in diabetic nephropathy (DN) pathogenesis. It has been shown that some inflammatory and fibrogenic pathways in the kidneys involve CD40 receptor/CD40 ligand (CD40L or CD154) signalling. Expression CD40 and CD40L has been found on a wide variety of cells, including lymphocytes and macrophages, endothelial, mesangial and tubular epithelial cells. CD40 expression on non-haematopoietic cells is upregulated in inflammatory contexts, suggesting that this receptor has an important role in vasculature and fibrosis. The aim of

our study was to assess the relationship between CD40-CD40L system and development of nephropathy in type 1 diabetic patients.

**Materials and methods:** 64 patients with type 1 diabetes, 32M/32F, age 16–49 years, were examined. 25 patients had normal albumin excretion rate (group DN0), 30 ones were microalbuminuric (group DN1) and 9 patients had macroalbuminuria (group DN2). The urinary excretion of CD40, soluble form of CD40L (sCD40L), as well as excretion of the molecules involved in inflammation and matrix remodeling, namely monocyte chemoattractant protein-1 (MCP-1), type IV collagen, transforming growth factor β1 (TGF-β1) and plasminogen activator inhibitor-1 (PAI-1), was determined by ELISA. Control group (C) comprised of 10 healthy subjects. Kidney biopsy was performed in 7 normoalbuminuric and 13 microalbuminuric patients. Data are presented as median, percentile 25–75.

**Results:** CD40 excretion was increased in all diabetic patient groups as compared to control (DN0: 157.1, 117.5–240.3, p=0.0006; DN1: 193.4, 144.5–222.3, p=0.0004; DN2: 337, 290.2–374.8, p=0.0004; C: 68.2, 37.6–105.8 pg/μmol creatinine). sCD40L excretion exceeded control in micro- and macroalbuminuric patients only (DN0: 0.16, 0.09–0.24, p=0.4; DN1: 0.22, 0.13–0.44, p=0.01; DN2: 0.23, 0.16–0.47, p=0.03; C: 0.12, 0.08–0.18 pg/μmol creatinine). CD40 and sCD40L correlated significantly with albuminuria (r=0.41 and r=0.42 respectively). CD40 excretion was related to the volume of renal interstitium (r=0.38), meanwhile sCD40L correlated with tubular basement membrane width (r=0.35). Patients with micro- and macroalbuminuria had increased excretion of MCP-1 (p=0.04 and p=0.005 respectively), type IV collagen (p=0.04 and p=0.0008), TGF-β1 (p=0.01 and p=0.004) and PAI-1 (p=0.02 and p=0.006). Significant correlations were found between CD40, sCD40L and excretion of MCP-1 (r=0.45 and r=0.42), type IV collagen (r=0.55 and r=0.34), TGF-β1 (r=0.5 and r=0.86) and PAI-1 (r=0.45 and r=0.9 respectively).

**Conclusion:** The activation of CD40/CD40L system is associated with development of nephropathy in type 1 diabetic patients. Inducing of chronic inflammation and inhibiting the extracellular matrix catabolism in the kidneys, CD40-CD40L interaction may contribute to development of renal fibrosis in diabetes.

Supported by: MD-5725.2010.7

## 1135

### Assessment of urinary L-FABP predictive value as a biomarker for diabetic nephropathy in type 1 diabetes

N.M. Panduru<sup>1,2</sup>, C. Forsblom<sup>2,3</sup>, L.M. Thorn<sup>2,4</sup>, M. Saraheimo<sup>2,4</sup>, A. Bierhaus<sup>5</sup>, P.M. Humpert<sup>6</sup>, P.-H. Groop<sup>3,2</sup>, on behalf of the FinnDiane Study Group;

<sup>1</sup>2nd Pathophysiology Department, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania, <sup>2</sup>Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, Finland, <sup>3</sup>Division of Nephrology, Department of Medicine, Helsinki University Central Hospital, Finland, <sup>4</sup>Helsinki University Central Hospital, Division of Nephrology, Department of Medicine, Finland, <sup>5</sup>Department of Medicine and Clinical Chemistry, University of Heidelberg, Germany, <sup>6</sup>Stoffwechselzentrum Rhein Pfalz, Mannheim, Germany.

**Background and aims:** Diabetic nephropathy (DN) has mainly been considered a glomerular disease, although tubular dysfunction may also play a role in its pathogenesis. Tubular markers like urinary L-FABP could thus be of potential interest for screening and diagnosis of DN. The aim of this study was to assess the predictive value and the potential clinical benefits of using this new marker at any stage of DN.

**Materials and methods:** At baseline, out of 2246 patients with type 1 diabetes 1549 had normal AER, 334 microalbuminuria and 363 macroalbuminuria. In addition, 208 non-diabetic subjects were studied. Patients were followed for a median of 5.8 years (95% CI 5.7 - 5.9) during which 112 patients progressed from normo- to microalbuminuria, 46 from micro to macroalbuminuria and 78 from macroalbuminuria to ESRD. Urinary L-FABP levels were measured by ELISA and normalized with u-creatinine, while other biochemical blood and urinary tests were performed by standard methods. Separate Cox proportional hazard models for the progression at every stage of DN were constructed and used to evaluate the predictive value of uL-FABP alone and after adjustment with AER. The clinical benefit of using uL-FABP alone or together with AER was assessed by ROC analyses.

**Results:** In a cross-sectional analysis, uL-FABP increased with worsening nephropathy stage (normo 0.039 vs micro 0.091 vs macro 0.504 μg/μmol, p<0.0001) and was higher in patients with normal AER than in non-diabetic subjects (0.0391 [95% CI 0.036 - 0.044] vs 0.0148 μg/μmol [0.007 - 0.020], p<0.0001). In Cox regression analyses, uL-FABP was an independent predic-

tor of progression at all stages of nephropathy. As would be expected, ROC curves for the prediction of progression (defined by albuminuria) were significantly larger for AER than for uL-FABP, except for patients with baseline macroalbuminuria, in whom the areas were similar. When we added uL-FABP on top of AER in the models, there was a trend towards improvement of risk prediction ( $p=0.09$ ) between  $AUC_{uL-FABP+AER}$  (0.786 [95% CI 0.765 - 0.807]) and  $AUC_{AER}$  (0.778 [0.756 - 0.799]) in normoalbuminuric patients.

**Conclusion:** Urinary L-FABP is a novel and independent predictor for progression of diabetic nephropathy irrespective of the disease stage. It may add some clinical benefit when used together with AER for identification of normoalbuminuric patients that will progress to microalbuminuria.

*Supported by: Folkhälsan Research Foundation, the Wilhelm and Else Stockmann Foundation*

## 1136

### Total plasmalogenes level in blood plasma and erythrocytes' membranes in patients with diabetic retinopathy

N. Akhrarova<sup>1,2</sup>, A. Tashmanova<sup>1,3</sup>, A. Alieva<sup>1,3</sup>, A. Tsoy<sup>1</sup>, Z. Shamansurova<sup>1,2</sup>, T. Saatov<sup>2,3</sup>,

<sup>1</sup>Republican Specialized Scientific-practical Medical Centre of Endocrinology, <sup>2</sup>Institute of Biochemistry of Academy of Sciences of the Republic of Uzbekistan, <sup>3</sup>Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan.

**Background and aims:** Diabetes mellitus (DM) is accompanied with carbohydrates, proteins, and lipids disturbances in blood and tissues. The recent studies showed decrease of the level of total plasmalogenes in blood plasma (BPP) and erythrocytes' membranes (EMP) in both type 1 (DM1) and type 2 DM (DM2) patients with diabetic peripheral neuropathy with increasing of DM severity. In this investigation we aimed to study the level of BPP and EMP in DM patients depending on severity of diabetic retinopathy (DR).

**Materials and methods:** The BPP and EMP level measured in 117 DM patients (63 with DM1 and 54 with DM2) and in 24 healthy subjects (HS). In all observed persons fasting and postprandial glycaemia, HbA<sub>1c</sub> level, total phospholipids level in plasma (PP) and erythrocyte membranes (PE) were measured. The BPP and EMP detection was based on measure of vinyl ether linkage. The DR degree was defined according to WHO criteria (2002) on ophthalmologist eye examination, where in 37 patients registered non-proliferative DR (DR1), in 31 - pre-proliferative DR (DR2), and in 26 - proliferative DR (DR3), and 23 patients with DM had no signs of DR (DR0).

**Results:** HbA<sub>1c</sub> level was increased in patients with DM1 (7.4%,  $p<0.01$ ) and DM2 (9.3%,  $p<0.001$ ) comparing to the HS (5.5%) suggesting poor control of diabetes. PP level did not differ from HS, while EP level was decreased in patients with DM1 on 9.8% ( $p<0.01$ ) and DM2 on 11.2% ( $p<0.001$ ) in comparison to HS suggesting lipids disturbances on cell plasma membrane. BPP and EMP level was significantly decreased comparing to HS on 49.8% ( $p<0.001$ ) and 53.9% ( $p<0.001$ ) in DM1, and on 20.0% ( $p<0.001$ ) and 19.8% ( $p<0.001$ ) in DM2, accordingly. The analysis found the negative linkage between DR severity and BPP and EMP levels, where at higher degree of DR, BPP and EMP levels were more decreased. BPP and EMP levels in patients with DR 2 and 3 were decreased on 6.0% and 7.0% comparing to DR0. This result together with recently obtained in patients with neuropathy also indicate the relationship between cell plasma membranes lipids abnormality and severity of diabetes complications.

**Conclusion:** In DM patients with increasing of DR severity BPP and EMP levels decreased suggesting association between plasma membranes lipids abnormalities and diabetes complications.

## PS 098 Diabetic nephropathy and retinopathy screening and mechanisms

### 1137

#### Longitudinal trends in use and costs of prescription medication in patients with type 1 diabetes and kidney transplant

R. Lithovius<sup>1,2</sup>, V. Harjutsalo<sup>1,2</sup>, C. Forsblom<sup>1,2</sup>, M. Saraheimo<sup>1,2</sup>, P. Koskinen<sup>2</sup>, P.-H. Groop<sup>1</sup>, FinnDiane Study Group;

<sup>1</sup>Institute of Genetics, Folkhälsan Research Center, <sup>2</sup>Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland.

**Background and aims:** Kidney transplantation is a cost-effective treatment for ESRD in patients with type 1 diabetes. Over the past decades new and more expensive patented drugs, including new immunosuppressants, have reached the markets. However, studies on the recent trends in medication use and costs among patients with type 1 diabetes and kidney transplant are sparse and thus, necessary to elucidate. Therefore, we estimated trends in prescription medication use and costs in patients with type 1 diabetes after kidney transplantation.

**Materials and methods:** A total of 330 type 1 diabetes patients with kidney transplant were identified from The Finnish Diabetic Nephropathy Study. The data were linked to the Drug Prescription Register (purchases of drugs 1995 - 2009), the Hospital Discharge Register (until 2009) and the Drug Reimbursement Register (until 2009). Patients were divided into two groups according to kidney transplantation year: Group 1: 1986 - 2000 [ $n=179$ , mean follow-up 8.3 ( $\pm 1.6$ ) years] and Group 2: 2000 - 2009 [ $n=151$ , mean follow-up 6.7 ( $\pm 2.0$ ) years]. The follow-up was divided into nine 12 months periods after the first month of the transplantation. Costs were inflated to 2009 euro levels by using the Consumer Price Index. The generalized linear mixed models were used to evaluate the drug costs during the 9-year time period after transplantation. Costs were adjusted for age, sex, BMI, co-morbidities and transplantation year.

**Results:** The total costs decreased by 28% in both groups (in Group 1 from €11,360 to €8,900 and in Group 2 from €12,620 to €9,820) during follow-up ( $p<0.0001$ ). The average decrease in Group 1 between the first and the second year was 11.7%, while it was 1.7% by every 12 months thereafter, and was 7.0% and 2.7% in Group 2, respectively. Although the costs profiles were similar between the two groups ( $p=0.9$  for time\*group interaction), the cost level was higher in Group 2 than in Group 1 ( $p<0.0001$ ). The decreasing trend was observed for the immunosuppressive drugs ( $p<0.0001$ ) in both groups. However, the cost level of these drugs was significantly higher in Group 2 ( $p<0.0001$ ) than in Group 1. The cost of diabetes drugs decreased slightly at the beginning of the follow-up, but stabilised thereafter. Other medication (immunosuppressive and diabetes drugs excluded) costs were stable ( $p=0.3$ ) during the whole time period. The proportion of immunosuppressive drugs was more than 70% of the total costs, while diabetes drugs counted less than 10%. In Group 1 the most common immunosuppressive regimen was cyclosporine, azathioprine and corticosteroid combination, while cyclosporine, mycophenolate mofetil with or without corticosteroid was the most common combination in Group 2.

**Conclusion:** The total costs of prescription medication decreased over time in patients with type 1 diabetes and kidney transplant. Since no considerable differences were observed in the costs of diabetes or other drugs, the observed trend was mainly due to the decrease in the costs of immunosuppressants. This finding is in accordance with the clinical practice guidelines to reduce doses of immunosuppressants over time to minimize side-effects.

*Supported by: Wilhelm and Else Stockmann Foundation*

## 1138

**Diabetes-related end stage renal disease in Austria 1965–2010 - analysis from the Austrian dialysis- and transplant registry**F.C. Prischl<sup>1</sup>, M. Auinger<sup>2</sup>, M.D. Säemann<sup>3</sup>, G. Mayer<sup>4</sup>, M. Wallner<sup>1</sup>, R. Kramar<sup>5</sup>;<sup>1</sup>Dept. Nephrology, Klinikum Wels-Grieskirchen, Wels, <sup>2</sup>3rd Dept. Internal Medicine, Krankenhaus der Stadt Wien-Hietzing, Vienna, <sup>3</sup>Dept. Nephrology and Dialysis, Medical University of Vienna, <sup>4</sup>Dept. Nephrology, Dialysis and Hypertension, Medical University of Innsbruck, <sup>5</sup>Austrian Dialysis and Transplant Registry, Wels, Austria.

**Background and aims:** As in other western countries diabetic kidney disease (DKD) is the leading cause of end-stage renal disease (ESRD) in Austria. There are marked differences in incidence and prevalence of DKD between countries even with comparable socio-economic status. We, therefore, analyzed ESRD due to DKD in Austria over 45 years and show that incident DKD-patients decrease since 2006.

**Materials and methods:** The Austrian Dialysis- and Transplant registry started in 1965 and yearly collects data on all patients with renal replacement therapy (RRT; including HD, PD, kidney alone and kidney combined with other organ transplantation). All patients with DKD-related ESRD (not diabetes as a comorbid condition) were analyzed longitudinally from 1965 until Dec 31<sup>st</sup>, 2010.

**Results:** Over 45 years, 7557 DKD-patients entered RRT programs in Austria (mean age at start: 63.1±12.6 years; 59.7% males; BMI 26.4±5.5 kg/m<sup>2</sup>). Mean RRT-duration amounted to 3.2±3.6 (median 2.0) years. Comorbid conditions reported any time were hypertension in 88.5%, cerebrovascular disease in 51.7%, coronary artery disease in 41.8%, peripheral atherosclerotic disease in 29.9%, and heart failure in 28.1% of patients. While DKD was not present in the ESRD-cohort in Austria until 1974, in 1975 seven incident type 1 diabetics and one type 2 diabetic started RRT (1.05 patients per million population - PMP). In the mid-eighties only, DKD increasingly necessitated RRT with a constant rise until 2006 (1985: 10.7 PMP; 1995: 33.6 PMP; 2005: 50.8 PMP; yearly growing rate 10%). After a peak of 436 incident DKD-ESRD patients in 2006, the numbers decreased continuously (2010: 350 patients, 41.7 PMP). This decrease in incident DKD-ESRD patients occurred despite a still growing diabetic population, estimated to amount to 390000 Austrians or 5.9 % of the population in 2007. Prevalence of all patients on RRT continuously increased from 755 PMP in 2001 to 995 PMP in 2010 and the same was true in patients with ESRD-DKD (2001: 100 PMP; 2010: 141 PMP). Mean age at RRT start was 35.0, 52.5, 62.4, 65.9 and 66.9 years in 1975, 1985, 1995, 2005 and 2010, respectively and on an average always was 3.1 years higher than in non-diabetics. According to the 2007 national survey on estimated diabetes prevalence, and compared to DKD patients commencing RRT the overall incidence rate PMP at risk was 135.2 compared to 1008.1 in diabetics with age-related marked differences. Finally, we calculated 5-year-survival probability in two DKD-cohorts, starting RRT in 1997-98 and in 2007-08. Survival was 28% in the period 1997-98 and 37.5% in the period 2007-08. Relative risk reduction unadjusted was 22% (HR 0.78, CI 95% 0.67-0.89; p=0.001).

**Conclusion:** Despite an increasing prevalence of diabetes in the Austrian population and in the RRT cohort, the incidence of DKD patients to enter RRT is decreasing since 2007 and 5-year survival probability is improving. It may be speculated that specific therapeutic maneuvers including antihypertensive therapy, especially ACE-inhibitors or ARBs, and multifactorial other therapeutic interventions may have resulted in this improvement.

## 1139

**Rate and determinants of association between retinopathy and nephropathy in patients with type 2 diabetes**G. Pugliese<sup>1</sup>, A. Solini<sup>2</sup>, G. Zoppini<sup>3</sup>, E. Orsi<sup>4</sup>, G. Zerbini<sup>5</sup>, R. Trevisan<sup>6</sup>, G. Gruden<sup>7</sup>, F. Cavalot<sup>7</sup>, L. Laviola<sup>8</sup>, S. Morano<sup>1</sup>, A. Nicolucci<sup>9</sup>, G. Penno<sup>2</sup>;<sup>1</sup>La Sapienza University, Rome, <sup>2</sup>University of Pisa, <sup>3</sup>University of Verona, <sup>4</sup>Fondazione IRCCS Cà Granda - Ospedale Maggiore Policlinico, Milan, <sup>5</sup>San Raffaele Scientific Institute, Milan, <sup>6</sup>Hospital of Bergamo, <sup>7</sup>University of Turin, <sup>8</sup>University of Bari, <sup>9</sup>Consorzio Mario Negri Sud, S. Maria Imbaro, Italy.

**Background and aims:** Few studies have investigated the association between diabetic retinopathy (DR) and nephropathy (DN) in type 2 diabetes. These studies examined small-sized samples and used only albuminuria or

proteinuria as markers of chronic kidney disease (CKD). This study was aimed at evaluating the rate and determinants of concordance between DR and CKD, as assessed by both albuminuria and estimated glomerular filtration rate (eGFR), in the large cohort of the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study.

**Materials and methods:** Patients with type 2 diabetes (n=15,773), visiting consecutively 19 hospital-based Diabetes Clinics throughout Italy in years 2007–2008, were examined. DR was assessed by funduscopy in mydriasis and patients were classified as having no, non-advanced DR (mild or moderate non-proliferative) or advanced DR (pre-proliferative, proliferative or maculopathy). CKD was defined based on albuminuria, as assessed by immunonephelometry or immunoturbidimetry, and eGFR was calculated from serum creatinine using the simplified Modification of Diet in Renal Disease Study formula. Patients were classified as having no CKD or the CKD phenotype with albuminuria, reduced eGFR or both.

**Results:** The majority of subjects (51.87%) had neither DR nor CKD. Concordance between DR and CKD was found only in 11.50% of patients. Of them, 50.22% had non-advanced and 49.78% had advanced DR, whereas 48.62% had albuminuria alone, 21.66% had reduced eGFR alone and 29.72% had both, thus indicating that DR is more frequent in the albuminuric than in the non-albuminuric CKD phenotypes. Overall, DR was found in 30.70% of the 5,908 individuals with any CKD, whereas CKD was detected in 51.87% of subjects with any DR and in 58.64% of those with advanced DR. Discordance between DR and CKD was observed in 36.63% of subjects, with 10.67% having only DR (62.15% non-advanced and 37.85% advanced) and 25.96% having only CKD (50.49% albuminuria alone, 31.27% reduced eGFR alone and 18.25% both). Age, male gender, diabetes duration, HbA<sub>1c</sub>, hypertension, triglycerides, previous cardiovascular disease (CVD) and, inversely, HDL cholesterol were independently associated with presence of any CKD in individuals with advanced DR; correlates differed according to the presence of albuminuria and/or reduced eGFR. Conversely, factors correlating with presence of advanced DR in subjects with any CKD were diabetes treatment, previous CVD, albuminuria, and, inversely, smoking, eGFR and age at diagnosis.

**Conclusion:** Concordance of DR with CKD is low in subjects with type 2 diabetes, and CKD without DR is more frequent than isolated DR, at variance with type 1 diabetes. Factors independently associated with presence of any CKD in individuals with advanced DR differ, at least partly, from those correlating with presence of advanced DR in subjects with any CKD and also according to the CKD phenotype.

*Clinical Trial Registration Number:* NCT00715481

*Supported by:* Fo.Ri.SID, DEM Foundation, Eli-Lilly, Takeda, Chiesi, Boehringer

## 1140

**Audit of avoiding over-referral to ophthalmology by monitoring early diabetic maculopathy within an inner city diabetes eye screening programme**

R.A. Cooper, G. Rayanagoudar, L.J. Bolter, P.N. Mitchell, J.V. Anderson; City, Hackney, Redbridge, Barking and Dagenham Diabetes Eye Screening Service, Homerton University Hospital, London, UK.

**Background and aims:** Photographic diabetic retinal screening relies on the analysis of two dimensional digital photographic images. These cannot detect the three dimensional abnormalities of macular oedema (M1). Image grading uses the presence of surrogate markers to infer the likely presence of macular oedema. These markers have high sensitivity but poor specificity. In the NHS Diabetic Eye Screening Programme this results in the referral of a large burden of patients into ophthalmology clinics, many of whom prove to need only observation. We defined a sub-category of M1 (M1a) and have audited the outcome of reviewing such cases 6-monthly within a diabetes eye screening service, rather than referring to ophthalmology.

**Materials and methods:** We defined M1a as: An image meeting the English National Grading criteria for M1, but with visual acuity (VA) of 6/6 or better (or 6/9 in the past with no deterioration when the new problem emerged) and with no new visual symptoms, unless the level of exudation is more than modest. Patients graded as M1a in one or both eyes between April 2008 and March 2009 were tracked through to their status at August 2011. Patients whose maculopathy worsened beyond the definition of M1a were referred to ophthalmology for assessment and treatment if necessary.

**Results:**

- 48 % of the 773 eyes either improved back to no maculopathy (M0) (30%) or remained stable at M1a (18%).
- 21% were referred not for worsening maculopathy in the index eye but for pre-proliferative (R2) or proliferative (R3) retinopathy in the eye with early



diabetic maculopathy (M1a) or referable retinopathy (R2, R3, M1) in the other eye.

- 7% of patients died, moved away or were lost to follow up
- Only 24% of the 773 eyes progressed to referable maculopathy during the follow up period of at least two and a half years.
- Of those referred for macular disease worse than M1a, only 1.81% of the cohort had a loss of 2 lines of vision or VA becoming worse than 6/12 at the point of referral.

Following referral for maculopathy worse than M1a, one third of patients have received focal macular laser photocoagulation in the follow up period. In one third of patients, the most recent VA was better than their VA at referral. In four fifths of eyes, VA had remained stable, dropped by fewer than two lines or had improved at recent follow up. More than four-fifths of eyes where the VA was better than 6/12 at referral were still above the driving threshold at recent follow up.

**Conclusion:** In whole population screening the monitoring of a pre-defined early stage of diabetic maculopathy within a diabetes eye screening service appears to offer a safe, convenient and low cost alternative reducing unnecessary referrals to ophthalmology.

## 1141

### Early detection of diabetic retinopathy by using digital fundus photography

M. Aguilar-Diosdado<sup>1</sup>, V. Regife<sup>2</sup>, E. Mayoral<sup>3</sup>, M. Cornejo<sup>3</sup>, S. Jimenez<sup>4</sup>, P. Alemany<sup>4</sup>, C. Lama<sup>2</sup>, L. Lahera<sup>2</sup>;

<sup>1</sup>Endocrinology & Nutrition Division, Puerta del Mar Hospital, Cadiz,

<sup>2</sup>Public Health Service of Andalusia, Sevilla, <sup>3</sup>Ophthalmology Division, Infanta Elena Hospital, Huelva, <sup>4</sup>Ophthalmology Division, Puerta del Mar Hospital, Cadiz, Spain.

**Background and aims:** Diabetic retinopathy (DR) is a principal cause of blindness in the developed countries and can be prevented by an early diagnosis and treatment. The aim of the study is to describe the results of the early detection of DR in the Andalusian Diabetic Retinopathy Early Detection Program (ADREDP).

**Materials and methods:** ADREDP is based in the DR screening by using digital fundus photography, performed at Primary Care centres and in Endocrinology offices. A first reading is performed by General Practitioners or Endocrinologists, who have been previously trained. In a second step, tests considered abnormal or doubtful are referred to the Ophthalmologist. As a false negative quality control, random and masked samples of tests considered as normal are referred for a second reading to an Ophthalmologist auditor.

**Results:** More than 2,000 care givers in 300 Primary Care centres and 36 hospitals have actively participated in this program. 215,000 subjects with diabetes have been included, and 254,200 tests have been finished: 82% were normal, 8% showed DR, 5% other findings and 5% were not valuations. Of 19,400 cases with DR, 94% were mild-moderate, 5% severe-very severe y 1% proliferative. 16.3% of the total tests were referred to the Ophthalmologist after the first reading because DR, and 2.3% because other pathological findings. In 3,500 tests considered as normal in the first study, the audit for the quality control found discrepancy in 10% (false negative).

**Conclusion:** The implementation of a global screening program of DR with digital fundus photography in a Public Health Service is useful, but an adequate planning and resources funding are needed. The high prevalence of mild and moderate grades of DR suggests it is a very suitable cost-benefit intervention.

Supported by: Institute of Health Carlos III and Andalusian Public Health Service

## 1142

### Distribution of diabetic retinopathy in the population attending the diabetic retinopathy screening service for Wales 2011-2012

A.F. Crowder<sup>1</sup>, G. Bhakta<sup>1</sup>, C.J. Richards<sup>1</sup>, R.L. Thomas<sup>2</sup>, S.D. Luzio<sup>2</sup>, R.A. Keigwin-Harris<sup>1</sup>, R. McPherson<sup>1</sup>, D.R. Owens<sup>2</sup>;

<sup>1</sup>Diabetic Retinopathy Screening Service for Wales, Rhondda Cynon Taf,

<sup>2</sup>Diabetes Research Group, Institute of Life Science, Swansea, UK.

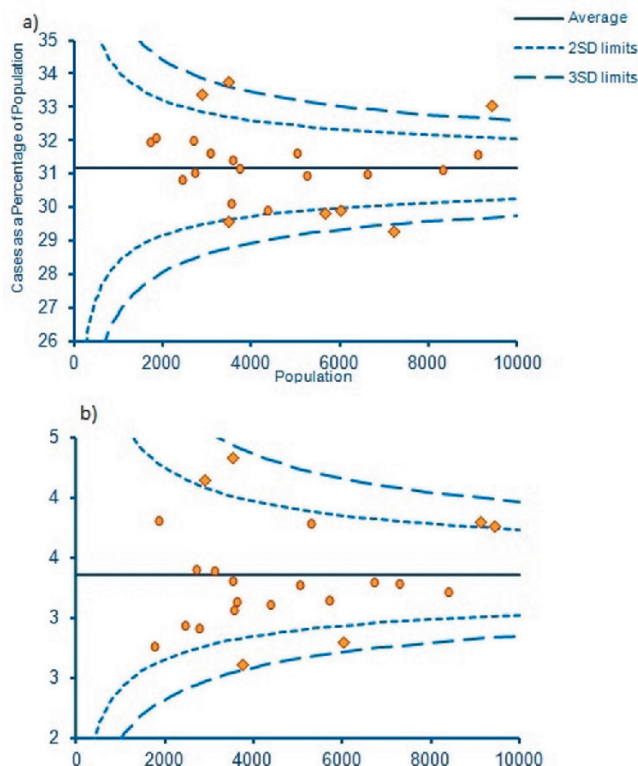
**Background and aims:** To determine the distribution of any diabetic retinopathy (DR) in the population attending for routine screening with the Diabetic Retinopathy Screening Service for Wales (DRSSW) during 2011.

**Materials and methods:** All eligible persons with diabetes (DM) in Wales over the age of 12 years attending a community based DR screening pro-

gramme between Jan 2011 and Jan 2012 were included in the analysis. Digital images were obtained using retinal photography (two 45° fields per eye) following mydriasis. Descriptive analysis were conducted utilising t-tests for continuous and chi-square for categorical variables.

**Results:** 102,427 persons (males 56.6%) with DM were screened of which 31,914 (31.1%) had DR. The percentage of any DR across the 22 localities is shown in Fig 1a. 15(68.1%) localities were within 2SD of the mean (31.2%  $\pm$ SD 1.2) (Group A), 4 (18.2%) had a lower (Group B) and 3 (13.6%) a higher (Group C) proportion of DR. Compared to A those in B and C were younger (66.5 vs 65.8 and 65.0 y, respectively  $p<0.001$ ). Compared to A those in B had fewer persons with T1DM (5.8 vs 5.4% ,  $p=0.02$ ), a higher proportion on diet (26.4 vs 28.5% ), and less on tablets (61.9 vs 60.4%) and insulin (11.7 vs 11.0%) ( $p<0.001$ ). Group C had more type 1 DM (6.3%,  $p=0.026$ ), fewer on diet (22.4%), and more on tablets (65.7%) and insulin (12.0%) ( $p<0.001$ ). The proportion of referable DR was within 2 SD's of the mean for 16 (72.7%) localities (Group X), with 2 (9.1%) having a lower (Group Y) and 4 (18.2%) a higher proportion (Group Z) (see Fig 1b). Compared to X those in Y and Z were younger (66.5 vs 65.9 and 65.1 y, respectively  $p<0.001$ ). Those in Y also had a lower proportion of T1DM (5.8 vs 5.3%  $p=0.047$ ). In comparison to X those in Z had a lower proportion on diet (27.4 vs 22.7%,  $p$  values), and a higher proportion on tablets (61.2 vs 65.2%) or insulin (10.6 vs 12.1%) ( $p<0.001$ ).

**Conclusion:** The majority of the 22 localities across Wales had similar proportions of any DR (15) and referable DR (16). Those with lower proportions had more persons with T2DM and on diet whilst those with higher proportions had more persons with T1DM and requiring insulin.



## 1143

### Associated factors for diabetic retinopathy in a Korean population: The 2008-2009 Korean national health and nutrition survey

D.-J. Kim, J. Yang, Y. Lee, J. Noh, J. Park, K. Ko, B. Rhee; Department of Internal Medicine, Inje University College of Medicine, Koyang, Republic of Korea.

**Background and aims:** We tried to evaluate the prevalence of and associated factors for diabetic retinopathy (DR) and investigate the optimal cutoff value of fasting plasma glucose (FPG) for DR in Korea.

**Materials and methods:** Among the 11,163 adults ( $\geq 19$  years old) from the fourth Korea National Health and Nutrition Examination Survey in 2008-2009, laboratory test, nutritional survey, and fundus examination data from

9,586 persons including 4144 men and 5472 women (age, 46 years [19–93]), were examined. For each participants, one 45 nonmydriatic digital retinal image centered on the fovea was taken per eye (2 images per person in total). DR was defined as the presence of 1 or more retinal microaneurysms or retinal blot hemorrhages with or without more severe lesions.

**Results:** Diabetes prevalence in this population was 8.6%. The prevalence of DR was 1.3% in whole population and that was 14.1% in persons with diabetes (3.9% in newly diagnosed diabetes, and 18.6% in previously diagnosed diabetes). In age- and sex-adjusted comparison, persons with DR were more obese and showed more frequency of hypertension, more frequency of diabetes, higher serum triglyceride, and lower serum HDL-cholesterol compared to those without DR. There was no difference of the presence of metabolic syndrome, self-reported cerebrovascular disease, or self-reported coronary heart disease according to the presence of DR. To evaluate associated factors for DR in whole population, logistic regression analysis for DR with age, sex, exercise, waist circumference, hypertension, serum HDL-cholesterol, serum triglyceride, anti-lipid drug, anti-diabetes medication, FPG, and diabetes duration as covariates was done. In this model, insulin treatment (vs. no anti-diabetes drug, OR [95% CI], 5.36 [1.93–14.90],  $p = 0.001$ ), FPG (1.01 [1.01–1.02],  $p < 0.001$ , per unit of FPG), and diabetes duration (1.07 [1.04–1.10],  $p < 0.001$ , per unit of diabetes duration) was associated with DR, respectively. Other parameters including age, sex, the presence of hypertension, and lipid profile were not significant. After including only persons with diabetes, insulin treatment, FPG, and diabetes duration was, also, associated with DR. To investigate the optimal cutoff value of FPG for DR, receiver operator curve analysis was done and 123.5 mg/dl of FPG was the optimal cutoff value (area under the curve = 0.876,  $p < 0.001$ ; sensitivity = 0.647, specificity = 0.970).

**Conclusion:** In this representative sample of Korean population, the prevalence of DR in persons with diabetes was about 14%, and the optimal cutoff value of FPG for DR was 123.5 mg/dl. The finding that only FPG and diabetes duration were associated with DR, suggested hyperglycemia could be the sole major determinant for DR and in the development of DR, other cardiovascular risk factors may be minor contributors.

## 1144

### Smoking as the potential link between Kimmelstiel-Wilson lesion and idiopathic nodular glomerulosclerosis: a single center retrospective study

R. Halmi, P. Degrell, I. Szejártó, G. Molnár, I. Wittmann;

2nd Department of Internal Medicine, University of Pécs, Medical Faculty, Hungary.

**Background and aims:** Nodular sclerosis is hallmark of both Kimmelstiel-Wilson lesion (KW) and idiopathic nodular glomerulosclerosis (ING). Chronic smoking is considered only as a risk factor of KW but as one potential cause of ING. The prevalence of smokers among patients with Kimmelstiel-Wilson lesion is unknown.

**Materials and methods:** In a retrospective analysis, native renal biopsy specimens ( $n=1128$ , 2001–2011) were evaluated, male patients' characteristics and smoking habits were assessed in three groups: diabetic patients either with KW ( $n=15$ ), or other classes of diabetic nephropathy (DNP;  $n=46$ ); and patients with ING ( $n=7$ ).

**Results:** Majority of the KW group (13/15= 87%) were smokers, unlike the DNP group (16/46= 35%;  $p=0.001$  vs. KW) but similar to the ING group (7/7= 100%;  $p=1.000$  vs. KW). However, the other known factors responsible for either the worsening of DNP or the development of ING did not differ in the groups (KW/DNP/ING; age, 56+/-1/ 56+/-9/ 55+/-1 ys;  $p=0.935$ ; body mass index, 30+/-5/ 31+/-5/ 28+/-6 kg/m<sup>2</sup>;  $p=0.538$ ; duration of diabetes mellitus, 11+/-6/10+/-7/0 ys;  $p=0.617$ ; glycaemic control (HbA<sub>1c</sub>, 6.5+/-1/6+/-1/- %;  $p=0.88$ ; prevalence of hypertension, 93/87/86 %;  $p=0.782$ ; and duration of hypertension, 11+/-8/ 13+/-10/ 11+/-6 ys;  $p=0.948$ ; serum total cholesterol, 6+/-4/ 6+/-2/ 7+/-2 mmol/l;  $p=0.500$ ; triglyceride, 3+/-3/ 3+/-3/ 3+/-1 mmol/l;  $p=0.784$ ; eGFR, 34+/-22/ 46+/-32/ 46+/-28ml/min;  $p=0.483$ ; and RAS-blocker treatment 100/ 87/100%;  $p=0.222$ ).

**Conclusion:** We propose that chronic cigarette smoking could contribute to the development of Kimmelstiel-Wilson lesions.

## PS 099 Predicting nephropathy

### 1145

#### The pulsatility index and the resistive index in peripheral arteries are associated with albuminuria excretion in type 2 diabetes

B. Yang, Z. Sun, H. Yin, S. Li, H. Jin, S. Wang, S. Qiu;

Diabetes Institute of Southeast University, Nanjing, China.

**Background and aims:** Diabetic nephropathy and peripheral arterial disease are common vascular complications among subjects with type 2 diabetes. The pulsatility index and the resistive index in renal arteries have been found to correlate with the severity of the renal disease. The objective of the present study was to investigate the potential relationship between these indices and the albuminuria excretion which may become effective markers for evaluating the association between peripheral artery disease and diabetic nephropathy in type 2 diabetes.

**Materials and methods:** The total number of 996 type 2 diabetes (522 females, 474 males, mean age 59 years) was enrolled in the study, excluding individuals with history of angiocardiopathy, cerebrovascular disease, end-stage renal disease, malignancy, amputation and overt calcification of the lower limbs (ankle brachial index > 1.3). Ankle brachial index was measured and calculated with Doppler ultrasound. The pulsatility index and resistive index were figured from the blood flow velocities by the measuring software. The maximum value of ankle brachial index and the average values of pulsatility index and resistive index of two legs were collected and analyzed individually. Parameter of urinary albumin excretion was evaluated from the albumin-to-creatinine ratio in a random spot sample, which at least 2 of 3 samples collected within 3- to 6-month time frame were used to decide the degree of albuminuria.

**Results:** In a multiple regression analysis, after adjustment with confounding factors such as age, sex, body mass index, heart rate, blood pressure, plasma glucose, plasma lipids and HbA<sub>1c</sub>, albumin-to-creatinine ratio correlated more significantly to the pulsatility index ( $r = 0.43$ ,  $P = 0.02$ ) and resistive index ( $r = 0.56$ ,  $P = 0.01$ ) than to the ankle brachial index ( $r = 0.12$ ,  $P = 0.04$ ). Furthermore, when dividing the patients into two groups by the median resistive index value, there was a magnificent distinction of albumin-to-creatinine ratio between the groups ( $P = 0.01$ ). For pulsatility index this difference was also present ( $P = 0.03$ ).

**Conclusion:** Pulsatility index and resistive index associated with the diabetic nephropathy, as reflected by the albumin-to-creatinine ratio during the early complication of diabetes. Pulsatility index and resistive index may be more suitable markers for evaluating the relationship between peripheral artery disease and diabetic nephropathy in type 2 diabetes.

### 1146

#### Bone mineral density, kidney function and arterial stiffness in long-standing type 1 diabetic patients with or without diabetic nephropathy

M. Lajer<sup>1</sup>, S. Theilade<sup>1</sup>, C. Joergensen<sup>1</sup>, F. Persson<sup>1</sup>, G. Andr sd ttir<sup>1</sup>, S.

Nielsen<sup>1</sup>, H. Reinhard<sup>1</sup>, H.-H. Parving<sup>2</sup>, L. Tarnow<sup>1</sup>, P. Lacy<sup>3</sup>, B. Williams<sup>3</sup>,

P. Rossing<sup>1</sup>;

<sup>1</sup>Steno Diabetes Center, Gentofte, Denmark, <sup>2</sup>Dep. of medical endocrinology, Rigshospitalet, Copenhagen, Denmark, <sup>3</sup>Dep. of Cardiovascular Sciences, Univ. of Leicester, UK.

**Background and aims:** Patients with chronic kidney disease including diabetic nephropathy have been suggested to have a lower bone mineral density (BMD). The aim of the study was to evaluate bone mineral density (BMD) and arterial stiffness among long-standing type 1 diabetic patients with or without overt diabetic nephropathy.

**Materials and methods:** Cross sectional evaluation of a prospectively followed cohort 141 patients with diabetic nephropathy (DN) (55% men; age [mean±SD] 53±9 years, 42±8 years of diabetes, duration of DN 22±6 years) and 230 with persistent normoalbuminuria (50% men, 58±10 years, 40±10 years of diabetes). Femoral and lumbar spine BMD (g/cm<sup>2</sup>) was measured by dual energy x-ray absorptiometry (DXA). Osteopenia and osteoporosis were defined by any T-score from -2.5 to -1.0 and <-2.5, respectively. Glomerular filtration rate (GFR) by <sup>51</sup>Cr-EDTA was determined in DN and estimated GFR (eGFR, MDRD formula) in normoalbuminuric patients. Pulse wave velocity (PWV) was measured by SphygmoCor and 24h pulse pressure (PP) by BPro watch.

**Results:** Among patients with DN, 73 (52%) and 36 (26%) had osteopenia or osteoporosis, respectively compared to 124 (54%) and 32 (14%) of normoal-

buminuric patients ( $p=0.013$ ). 34% of males with DN vs. 14% with normoalbuminuria had osteoporosis ( $p<0.001$ ) and lower age-sex matched Z-scores ( $p<0.001$ ). Among women, only femoral Z-scores were different. Baseline eGFR levels did not change with declining BMD. Among patients with DN, 24h brachial and central PP were elevated ( $p=0.007$  and  $0.014$ , respectively) while 24h brachial and central systolic dip were lower in osteoporosis patients compared to osteopenia and normal BMD ( $p<0.001$ ). PWV also tended to be elevated and GFR lower in osteoporosis patients although not significantly ( $p=0.12$  and  $0.14$ ). Among normoalbuminuric patients, both 24h brachial and central BP and PWV were elevated in osteoporosis patients ( $p=0.004$ ,  $0.005$  and  $0.028$ , respectively) while 24h brachial and central systolic dip were not significantly different.

**Conclusion:** The risk of osteoporosis was highest among male type 1 diabetes patients and long-standing DN and femoral BMD was associated with albuminuria status, pulse pressure and dipping. Hence, screening and treatment of osteoporosis in patients with albuminuria or arterial stiffness should be considered.

## 1147

### Impaired relaxation of arterial wall during a rest in supine position may be a risk factor of arteriosclerosis and it is frequent in diabetic patients

H. Sasaki<sup>1</sup>, S. Kurisu<sup>1</sup>, J. Ibatani<sup>1</sup>, M. Nakatani<sup>1</sup>, K. Ogawa<sup>1</sup>, H. Yamasaki<sup>1</sup>, H. Wakasaka<sup>1</sup>, H. Furuta<sup>1</sup>, M. Nishi<sup>1</sup>, T. Akamizu<sup>1</sup>, K. Nanjo<sup>2</sup>

<sup>1</sup>First Department of Medicine, Wakayama University of Medical Science,

<sup>2</sup>Wakayama Rosai Hospital, Japan.

**Background and aims:** Although hyperactivity of adrenergic function has been supposed to promote arteriosclerosis, simple clinical methods to assess early adrenergic dysfunction did not exist. Cardio-ankle vascular stiffness index (CAVI) is a newly established method for estimating arterial wall stiffness which reflects both of elastic and muscular arteries. CAVI has been reported to decrease by the administration of alpha-blockade. Therefore, CAVI may reflect the change of vascular stiffness mediated by alpha-adrenergic vasomotor function. Our aim was to examine whether the change of CAVI during a resting reflects adrenergic function or not, and another aim was to elucidate the association between the change of CAVI and arteriosclerotic markers in non-diabetic (NDM) and diabetic (DM) subjects.

**Materials and methods:** *1st study:* We measured CAVI twice just at coming the laboratory and after 20 minutes' rest in supine position in 82 NDM and 40 DM subjects. The changes of CAVI (d-CAVI) were calculated and compared between NDM and DM subjects. The relationship between d-CAVI and the intima-media thickness of carotid artery (IMT) measured by ultrasound sonography was investigated in 38 NDM and 22 DM subjects. The clinical risk factors associated to IMT were evaluated by the multiple regression analysis using IMT as a dependent variable, gender, BMI, systolic blood pressure, HbA1c, d-CAVI as independent variables. *2nd study:* We assessed the change of adrenergic vasomotor tone during cold stimulation by continuous monitoring of photoplethysmogram wave at contralateral index finger and big toe in 49 NDM subjects. Cold stimulation was performed by the immersion of left hand into ice-water for 10 seconds. The subjects were divided into two groups according to the vasoconstriction response, good response (GR) group and poor response (PR) group. CAVI was also measured twice before and after the cold stimulation, and the changes of CAVI were compared between GR and PR groups.

**Results:** *1st study:* Though CAVI of NDM subjects decreased significantly by rest in supine position ( $6.69 \rightarrow 6.42$ ,  $p<0.001$ ), CAVI of DM patients did not significantly change ( $8.50 \rightarrow 8.32$ ,  $p=0.143$ ). Prevalence of the subjects whose CAVI were not decreased bilaterally in DM patients was significantly higher than that in NDM subjects ( $63\%$  vs  $43\%$ ,  $p=0.039$ ). D-CAVI was significantly correlated with IMT in NDM ( $r=0.374$ ,  $p=0.020$ ), but not in DM subjects. When NDM subjects were divided into 3 groups by d-CAVI equally, the IMT of smaller tertile was significantly thicker than that of larger tertile ( $0.61$  mm vs  $0.45$  mm,  $p=0.009$ ). Multiple regression analysis revealed that significant risk factors for thicker IMT were BMI and d-CAVI in NDM subjects. *2nd study:* GR and PR groups consisted of 24 and 25 subjects, respectively. Though CAVI increased by cold stimulation significantly in GR group ( $6.08 \rightarrow 6.35$ ,  $p=0.012$ ), CAVI did not significantly changed in PR group ( $6.58 \rightarrow 6.77$ ,  $p=0.077$ ).

**Conclusion:** Our data suggest that arterial wall relaxation by resting could be evaluated by serial CAVI measurement, and that arterial wall relaxation might reflect adrenergic vasomotor function, and that impaired relaxation is more frequent in DM than NDM subjects and it is significantly associated with arteriosclerosis.

## 1148

### Cerebral perfusion reserve and endothelial function in patients with type 1 diabetes mellitus

E. Kosobyan<sup>1</sup>, I. Jarek-Martynowa<sup>1</sup>, M. Martinov<sup>2</sup>, M. Shestakova<sup>1</sup>;

<sup>1</sup>Endocrinology Research Center, <sup>2</sup>Russian National Research Medical University named after N.I. Pirogov, Moscow, Russian Federation.

**Background and aims:** To examine cerebral perfusion reserve (CPR) and endothelial function in patients with a type 1 diabetes mellitus (T1DM) and microangiopathy.

**Materials and methods:** We examined 130 patients with T1DM (66 women and 64 men), age from 18 to 40 years (mean age  $28.3 \pm 4.6$  years), mean duration of T1DM  $17.1 \pm 6.1$  years. The control group (CG) included 26 healthy persons matched by gender and age. 72 (55.4%) patients had diabetic nephropathy (DN): Chronic Kidney Disease stage 1 (CKD1) - 25 (19.2%), Chronic Kidney Disease stage 2 (CKD2) - 22 (16.9%), Chronic Kidney Disease stage 3 (CKD3) - 25 (19.2%). Diabetic retinopathy (DR) was diagnosed in 110 persons: nonproliferative retinopathy (NPR) - 47 (36.6%), preproliferative retinopathy (PPR) - 22 (16.9%), proliferative retinopathy (PR) - 41 (31.5%). Groups of patients with T1DM were comparable on level of HbA1c. Parameters of blood flow velocity in the middle cerebral artery (MCA) were assessed by Transcranial Doppler ("Bioss", Russia). To determine the CPR we used carotid compression test. Arterial stiffness index (SI) and endothelial function was assessed by peripheral arterial tonometry technique ("Angioscan", Russia).

**Results:** Indicators of CPR in patients with CKD0 ( $12.9 \pm 13.0\%$ ) did not differ significantly from the CG ( $23.0 \pm 7.2\%$ ,  $p=0.06$ ). CPR was reduced in comparison with DR0 ( $9.2 \pm 6.1\%$ ,  $p=0.019$ ). There was a reduction of CPR in patients with DN ( $6.0 \pm 7.7\%$ ,  $p<0.001$ ) compared with CG; and reduction of CPR in patients with DR ( $9.2 \pm 11.6\%$ ,  $p=0.019$ ) compared with CG. In patients with no signs of DR we observed an increase in peak systolic blood flow velocity in the MCA ( $95.5 \pm 10.3$  m/s) compared with the CG ( $72.0 \pm 10.4$  m/s,  $p=0.047$ ). SI were significantly higher in the group of patients with diabetes ( $9.5 \pm 2.6$  m/s) in comparison to CG ( $8.3 \pm 1.3$  m/s,  $p=0.028$ ). When comparing SI between patients with T1DM without microvascular complications and CG we observed no statistically significant difference. SI was higher in patients with NPR ( $9.5 \pm 1.8$  m/s,  $p=0.02$ ), PPR ( $9.8 \pm 2.6$  m/s,  $p=0.041$ ), PR ( $10.4 \pm 2.0$  m/s,  $p<0.001$ ) compared with healthy subjects ( $8.3 \pm 1.3$  m/s) and CKD1 ( $9.7 \pm 2.1$  m/s,  $p<0.001$ ), CKD2 ( $11.2 \pm 2.8$  m/s,  $p=0.034$ ), CKD3 ( $9.8 \pm 1.8$  m/s,  $p<0.001$ ). During reactive hyperemia test: increase in the amplitude of signal prevailed over decline in the progression of DR ( $r=0.86$ ,  $p=0.003$ ), DN ( $r=0.99$ ,  $p=0.001$ ).

**Conclusion:** The index SI may be an early marker of macrovascular diseases in patients with microvascular complications. There is a decrease of the autoregulatory capacity of cerebral arteries in patients with T1DM, which increases with the development of microvascular complications. Increased peak systolic blood flow velocity in the MCA in the absence of DR may be a sign of the beginning of formation of changes in the retina.

## 1149

### Clinical evaluation of analytical variations in serum creatinine measurements: why laboratories should abandon Jaffé techniques

I. Drion<sup>1</sup>, N. Kleefstra<sup>1</sup>, K.H. Groenier<sup>1</sup>, C. Cobbaert<sup>2</sup>, C. Weykamp<sup>3</sup>, J.F.M. Wetzels<sup>4</sup>, H.J.G. Bilo<sup>1</sup>;

<sup>1</sup>Diabetes Centre, Zwolle, <sup>2</sup>Clinical Chemistry, Leiden University Medical Center, Zwolle, <sup>3</sup>European Reference Laboratory, Streekliekenhuis Koningin Beatrix, Winterswijk, <sup>4</sup>Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.

**Background and aims:** Serum creatinine (SCr) based prediction equations are frequently used in screening and clinical settings in order to assess the glomerular filtration rate (GFR). Moreover, chronic kidney disease (CKD) staging directly relies on these estimated GFR values. Using accurate SCr measurements is essential, since systematic errors cause unreliable renal function estimates, leading to incorrect drug dose adjustments, misclassifications in CKD staging and incomparability of patient data [1-4]. Non-equivalence in serum creatinine (SCr) measurements across Dutch laboratories and the consequences hereof on chronic kidney disease (CKD) staging were examined.

**Materials and methods:** National data from the Dutch annual external quality organization of 2009 were used. 144 participating laboratories examined 11 pairs of commutable, value-assigned (by the Joint Committee on Traceability in Laboratory Medicine) SCr specimens in the range  $52\text{--}262$   $\mu\text{mol/L}$ .



using Jaffé or enzymatic techniques. Regression equations were created for each participating laboratory (by regressing values as measured by participating laboratories on the target values of the samples sent by the external quality organization); area under the curves were examined and used to rank laboratories. The 10<sup>th</sup> and 90<sup>th</sup> percentile regression equation were selected for each technique separately. To evaluate the impact of the variability in SCr measurements and its eventual clinical consequences in a real patient population, we used a cohort of 82424 patients. The SCr measurements of these 82424 patients were introduced in the 10<sup>th</sup> and 90<sup>th</sup> percentile regression equations. The newly calculated SCr values were used to calculate an estimated glomerular filtration rate (eGFR) using the 4-variable Isotope Dilution Mass Spectrometry traceable Modification of Diet in Renal Disease formula. Differences in CKD staging were examined, comparing the stratification outcomes for Jaffé and enzymatic SCr techniques.

**Results:** Jaffé techniques overestimated SCr: 21%, 12%, 10% for SCr target values 52, 73 and 94  $\mu\text{mol/L}$ , respectively. For enzymatic assay these values were 0%, -1%, -2%, respectively. eGFR using the MDRD formula and SCr measured by Jaffé techniques, staged patients in a lower CKD category. Downgrading to a lower CKD stage occurred in 1-42%, 2-37% and 12-78.9% of patients for the 10<sup>th</sup> and 90<sup>th</sup> percentile laboratories respectively in CKD categories 45-60, 60-90 and >90 mL/min/1.73m<sup>2</sup>. Using enzymatic techniques, downgrading occurred only in 2-4% of patients.

**Conclusion:** Enzymatic techniques lead to less variability in SCr measurements than Jaffé techniques, and therefore result in more accurate staging of CKD. Therefore the specific enzymatic techniques are preferably used in clinical practice in order to generate more reliable GFR estimates.

## 1150

### The performance of MDRD and CKD-EPI equations in diabetic patients with various degrees of albuminuria

M. Vucic Lovrencic<sup>1</sup>, V. Radisic Biljak<sup>1</sup>, M. Prasek<sup>2</sup>, P. Pavkovic<sup>3</sup>, M. Knotek<sup>3</sup>;

<sup>1</sup>Institute of Clinical Chemistry and Laboratory Medicine, Merkur Teaching Hospital, <sup>2</sup>Vuk Vrhovac University Clinic, Merkur Teaching Hospital,

<sup>3</sup>Institute of Nephrology, Merkur Teaching Hospital, Zagreb, Croatia.

**Background and aims:** Early changes in diabetic nephropathy involve increased urinary albumin excretion rate (AER) and/or a temporal increase in glomerular filtration rate (GFR, hyperfiltration), which are not necessarily inter-related. Both albuminuric and non-albuminuric pathways to renal impairment have been identified in diabetes, emphasizing the importance of monitoring appropriate markers when screening for diabetic nephropathy. The aim of this study was to compare the performance of Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease Study (MDRD) equation in estimating GFR in a cohort of diabetic patients with various degrees of albuminuria.

**Materials and methods:** In a group of 842 diabetic patients GFR was estimated from standardized creatinine, with MDRD-Study and CKD-EPI equations, and their performance evaluated regarding clinical stages categories of albuminuria and chronic kidney disease (CKD).

**Results:** A total of 842 adult diabetic patients (48,2% males, 82% type 2 diabetes) were included in this study. Females were younger (median age: 55 vs 58,  $P=0.013$ ), had lower serum creatinine ( $65\pm14$  vs  $80\pm18$   $\mu\text{mol/L}$ ,  $P<0.001$ ), but no gender-associated differences in eGFR, calculated by either equation, HbA<sub>1c</sub> and albuminuria were found. Patients with normoalbuminuria ( $n=364$ ) had higher eGFR when calculated by CKD-EPI, than MDRD-Study equation [median (IQR): 103 (91-115) vs 97 (85-113) mL/min/1.73 m<sup>2</sup>,  $p=0.006$ ,  $n=364$ ], which significantly influenced the prevalence of stage 1 CKD [eGFR>90 mL/min/1.73 m<sup>2</sup>: 76,7% (CKD-EPI) vs. 65,1% (MDRD-Study),  $P=0.005$ ]. There were no differences between the eGFR values derived by two equations in patients with micro- and macroalbuminuria ( $n=290$  and 198, respectively), and more advanced staging of CKD.

**Conclusion:** CKD-EPI equation might be a superior surrogate marker of GFR in patients with normoalbuminuria and hyperfiltration and could be used as a screening tool for early renal impairment in diabetes. Its validity as a marker of progression of diabetic nephropathy remains to be established.

## 1151

### The effects of RAAS blockade on CIAKI (contrast induced acute kidney insufficiency) in patients with type 2 diabetes and in non-diabetic patients

E. Lee<sup>1</sup>, M. Kim<sup>1</sup>, N. Han<sup>1</sup>, S. Kim<sup>1</sup>, T. Kim<sup>1</sup>, T. Kim<sup>1</sup>, M. Kwon<sup>1</sup>, S. Lee<sup>1</sup>, M. Park<sup>2</sup>, B. Rhee<sup>1</sup>, J. Park<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, College of Medicine, Inje University,

<sup>2</sup>Department of Internal Medicine, College of Medicine, Dong-A University, Busan, Republic of Korea.

**Background and aims:** An ACE inhibitor or ARB is a drug used primarily for the treatment of hypertension in patients with diabetes mellitus because of effect of proteinuria reduction. CIAKI (contrast induced acute kidney insufficiency) is defined as an increase in serum creatinine within 72 hours after the contrast administration in the procedure using of contrast medium, such as coronary angiography. RAAS blockade may blunt autoregulation of kidney and precipitate prerenal ARF in patients with volume depletion. The CIAKI is also caused by a similar mechanism. But, there are few study about their relationship. The purpose of this study was to compare the influence of ACE inhibitors or ARBs on CIAKI in patients with type 2 diabetes and in non-diabetic patients.

**Materials and methods:** A total of 1472 patients who undergoing coronary angiography was divided into four groups according to hystory of diabetes and previous use of ACE inhibitor or ARB, then the incidence of CIAKI in each group were compared. CIAKI was defined as an increase in serum creatinine of  $\geq 25\%$  or  $\leq 0.5\text{mg/dl}$  above the baseline value within 72 hours after the contrast administration. In the analysis, patient outcoms were adjusted for age, sex, before creatinine, smoking status, dye volume, haemoglobin and eGFR and compared using propensity score-matched analysis.

**Results:** RAAS blockade(+) group showed higher incidence of CIAKI compared to RAAS blockade (-) group, but there were other compounding factors. To overcome limitations, we performed analysis after matching groups using propensity score. Among non-diabetic patients, the incidence of CIAKI was 22.9% in the RAAS blockade (+) group and 14.0% in RAAS blockade (-) group. The odds ratio for development of CIN with respect to RAAS blockade was 1.821( $P=0.018$ , 95% CI, 1.104-3.005). In diabetic patient group, the incidence of CIAKI was 16.0% in the RAAS blockade (+) group and 13.6% in RAAS blockade (-) group. The odds ratio for development of CIN with respect to RAAS blockade was 1.217( $P=0.532$ , 95% CI, 0.658-2.250). They showed same direction and tendency under sensitivity analysis.

**Conclusion:** Although use of ACE inhibitor or ARB may be an independent risk factor for developing CIAKI in non-diabetic patients, no harmful effect was found in diabetic patients.

## 1152

### Prevention of contrast-induced nephropathy in diabetic patients with renal function impairment: sodium bicarbonate versus sodium chloride-based hydration

P. Boucek, T. Havrdova, O. Oliyarnyk, J. Skibova, V. Pecenkova, D. Sarkady; Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

**Background and aims:** Contrast-induced nephropathy (CIN) is defined as renal function impairment following intravascular contrast medium use and hypothesized i.a. to be mediated by oxidative stress in the acid environment of the renal medulla. Sodium bicarbonate ( $\text{NaHCO}_3$ ) hydration has been used as preventive measure in studies of CIN, but its positive protective effects remain controversial. Moreover, the utility of  $\text{NaHCO}_3$  in patients with diabetes (DM) and kidney disease, a population at major risk of CIN, has not been addressed specifically, but merely as subgroup analysis.

**Materials and methods:** We present data from a prospective, randomized, double blind, sodium chloride (NaCl) hydration-controlled study of  $\text{NaHCO}_3$  which included 120 DM patients with impaired renal function (screening creatinine  $\geq 100$   $\mu\text{mol/L}$  (1.1 mg/dL)) undergoing an elective radiologic procedure with contrast medium use. Patients were randomized to receive a  $\text{NaHCO}_3$  ( $n=61$ ) or NaCl ( $n=59$ ) in dextrose infusion (154 mmol/L; 3 mL/kg/hour for 1 hour before and 1 mL/kg/hour for 6 hours after procedure). The primary end-point - incidence of CIN - was defined as creatinine increase of  $\geq 25\%$  and/or  $\geq 44$   $\mu\text{mol/L}$  (0.5 mg/dL) within 2 days following administration of contrast. Secondary end-points were maximal changes in creatinine, eGFR (MDRD formula) and spot urine 8-isoprostane levels (8-IPF<sub>2a</sub>/creatinine ratio) as measure of oxidative stress.

**Results:** Apart from marginally lower age of the  $\text{NaHCO}_3$ -treated patients (mean (SD); 63 (11) vs. 67 (10) yrs;  $p=0.49$ ), the groups were comparable with

regard to sex, BMI, HbA<sub>1c</sub>, baseline creatinine, eGFR, glycaemia and contrast dose given. CIN occurred in 7 (11.5 %) and 5 (8.5 %) patients of the NaHCO<sub>3</sub> and NaCl groups, resp. ( $p=0.76$ ; Fisher's exact test). No significant difference was seen in any of the secondary outcome measures. (Table).

Group	creatinine ( $\mu\text{mol/L}$ )	$\Delta$ creatinine ( $\mu\text{mol/L}$ )	eGFR ( $\text{mL/s}$ )	$\Delta$ eGFR ( $\text{mL/s}$ )	8-iPF <sub>2a</sub> / creatinine ( $\text{pg/mg}$ )	$\Delta$ 8-iPF <sub>2a</sub> / creatinine ( $\text{pg/mg}$ ) <sup>*</sup>
NaHCO <sub>3</sub>	170 (84)	14 (31)	0.69 (0.3)	-0.06 (0.08)	937 (506)	36 (525)
NaCl	160 (74)	9 (26)	0.70 (0.27)	-0.05 (0.08)	1081 (661)	19 (749)
P	0.49	0.33	0.84	0.45	0.20	0.89

Comparison of baseline values and maximal changes ( $\Delta$ ) within 2 days post contrast (two-sample t-test)

Means (SD); <sup>\*</sup>Full data available in 53 patients in each group

**Conclusion:** Though limited by its single-centre design, the results of the study suggest that sodium bicarbonate does not provide additional protection against contrast-induced nephropathy in DM patients with impaired renal function in comparison with standard sodium chloride-based hydration. *Clinical Trial Registration Number: EudraCT: 2007-007546-36*  
*Supported by: Grant MZO 00023001 of the Czech Ministry of Health*

## 1153

### Analysis on the prevalence and risk factors of abnormal fasting glucose after renal transplantation

M. Yu<sup>1</sup>, M. Chen<sup>1,2</sup>, M. Xu<sup>3</sup>, J. Gao<sup>4</sup>, X. Gao<sup>1</sup>;

<sup>1</sup>Endocrinology, Zhongshan hospital affiliated to Fudan University, Shanghai, <sup>2</sup>Endocrinology, Fujian Provincial people's Hospital, Fujian, <sup>3</sup>Urology, Zhongshan hospital affiliated to Fudan University, Shanghai, <sup>4</sup>Evidence-Based Medicine Center, Zhongshan hospital affiliated to Fudan University, Shanghai, China.

**Background and aims:** To explore the abnormal rate of PTDM diagnosed based on FPG and its associated risk factors in patients surviving for more than 1 year after renal transplantation.

**Materials and methods:** For 428 non-diabetic patients before transplantation receiving kidney transplant between 1 January, 1993 and 31 December, 2008, we analyzed the prevalence and associated risk factors of PTDM after transplantation according to FPG.

**Results:** Of the 428 Patients, 87 developed PTDM (20.3%) within a mean follow-up of (5.65 $\pm$ 3.68) years. The onset of PTDM occurred in 57 patients (in 65.5% of total PTDM) primarily within the first year after transplantation. There was no difference in the incidence of PTDM among three transplantation ages. Univariate analysis identified variables to be associated with the onset of PTDM: older age, a higher BMI, smoking history, positive family history of diabetes mellitus, deceased donor transplantation, hepatitis C virus infection, cytomegalovirus infection, FPG pre-transplantation as well as 1 week after transplantation, TC and TG pre-transplantation, a switch from CSA to FK506-based immunosuppressive regimen, peak plasma concentration of CSA in the first 6 and 12 months. The prevalence of PTDM were markedly elevated ( $P<0.05$ ) in the group who were converted to FK506 but not in the group who were converted to RAM. By multivariate analysis, five factors were independently associated with the onset of PTDM: FPG pre-transplantation (OR=1.48,  $P=0.036$ ), older recipient age (OR=1.10,  $P=0.044$ ), BMI (OR=1.18,  $P=0.035$ ).

**Conclusion:** The incidence of PTDM diagnosed based on FPG was 20.3% in patients surviving for more than 1 year after renal transplantation, and the onset of PTDM occurred primarily (65.5% of total PTDM) within the first year after transplantation. Main independent risk factors of PTDM included higher FPG levels, older recipient age, BMI, hepatitis C virus infection, deceased donor transplantation.

## PS 100 Small fibres in neuropathy: clinical

### 1154

#### SUDOSCAN: a rapid, objective and non-invasive method of diagnosing diabetic autonomic neuropathy

S. Tesfaye, J. Daives, A. Sankar, T. Cash, E. Cachia, G. Rao, D. Selvarajah; Diabetes Research Unit, University of Sheffield, UK.

**Background and aims:** Diabetic cardiac autonomic neuropathy (CAN) is a serious complication of diabetes and carries up to a five-fold increased risk of mortality. Current methods of detecting CAN are based on a battery of cardiovascular reflex tests (CARTs) that are too cumbersome and impractical for widespread use in busy clinical practice. SUDOSCAN is a new and rapid method of assessing sudomotor function by measuring electrochemical skin conductance (ESC) through reverse iontophoresis. The aim of the present study was to evaluate if SUDOSCAN can reliably diagnose CAN.

**Materials and methods:** Healthy, non-diabetic volunteers (HV,  $n=13$ ) 41 and subjects with type 1 diabetes ( $n=41$ ) and 13 healthy volunteers (HV) underwent detailed assessments including clinical, neurophysiological (Neuropathy Impairment Score of the Lower Limbs NISLL+7 tests of nerve function [NISLL+7]) and 5 CARTs (O'Brien protocol). Based on CARTs subjects were divided into CAN [3 abnormal CART results], Subclinical CAN [1-2 abnormal results] and No-CAN [0 abnormal results]. All subjects underwent the SUDOSCAN test. Participants placed their hands and feet on electrodes, and an incremental low direct current ( $<4\text{V}$ ) was applied to the anode for 2min. ESC ( $\mu\text{Siemens}$ ) as a measure of sudomotor function was obtained from the voltage and current and an autonomic risk score was calculated.

**Results:** The results presented in the table below show that SUDOSCAN can detect established CAN having corrected for age and BMI.

**Conclusion:** SUDOSCAN a simple, rapid ( $<5\text{min}$ ) and objective method of screening that doesn't require special patient preparation or medical personnel training may serve as screening test for CAN. It could help adhere to ADA's recommendation of annual AFT for all diabetic patients which is currently not fulfilled as current tests are cumbersome and time consuming.

	HV	No-CAN	Subclinical CAN	CAN	p-value*	p-value adj.**
N	13	13	15	13	-	-
Gender (male, n)	9	5	7	9	-	-
Age (years)	41 $\pm$ 18	43 $\pm$ 13	55 $\pm$ 16	50 $\pm$ 14	0.068	-
BMI (kg/m <sup>2</sup> )	23.9 $\pm$ 3.7	28.4 $\pm$ 5.9	29.6 $\pm$ 5.7	30.3 $\pm$ 6.0	0.018	-
Systolic blood pressure (mm Hg)	132 $\pm$ 18	140 $\pm$ 11	140 $\pm$ 14	139 $\pm$ 17	0.503	0.409
ESC hands ( $\mu\text{S}$ )	62 $\pm$ 15	59 $\pm$ 12	62 $\pm$ 18	61 $\pm$ 15	0.893	0.886
ESC feet ( $\mu\text{S}$ )	78 $\pm$ 6	76 $\pm$ 6	78 $\pm$ 9	60 $\pm$ 26	<b>0.003</b>	<b>0.004</b>
ESC feet/hands ratio	1.3 $\pm$ 0.3	1.4 $\pm$ 0.3	1.3 $\pm$ 0.3	1.0 $\pm$ 0.4	<b>0.045</b>	<b>0.044</b>
Autonomic risk score (%)	22 $\pm$ 14	30 $\pm$ 15	37 $\pm$ 18	39 $\pm$ 13	<b>0.022</b>	<b>&lt;0.001</b>

\* One way ANOVA

\*\* ANCOVA adjusted on age and BMI

## 1155

#### Determination of diabetic peripheral neuropathy prevalence and associated risk factors in Chinese diabetic and pre-diabetic subjects

B. Lu, J. Hu, J. Wen, Y. Li;

Department of Endocrinology, Huashan Hospital, Institute of Endocrinology and Diabetology at Fudan University, Shanghai, China.

**Objective:** Diabetes is a major risk factor during the development of neuropathy. We aimed to assess the prevalence of diabetic peripheral neuropathy (DPN) in community-based population and risk factors associated with DPN in type 2 diabetic patients.

**Research design and method:** An urban community-based sample of 1099 adults in Shanghai was classified into normal glucose tolerance (NGT), impaired glucose regulation (IGR), Newly-diagnosed diabetes mellitus (NDM) and known diabetes mellitus (KDM). All the subjects were evaluated on complete foot examination. DPN was assessed by using neuropathy symptom score (NSS) and neuropathy disability score (NDS). Body mass index (BMI), waist hip ratio (WHR), fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG), glycated haemoglobin (HbA<sub>1c</sub>), blood lipid and urinary albumin-to-

creatinine ratio (ACR) were investigated. DPN was defined as NDS $\geq$ 3 and NSS $\geq$ 5, or NDS $\geq$ 6. Data were compared among groups.

**Results:** The prevalence of DPN was 12.7% and 3.30% in the KDM and IGR patients respectively. The prevalence of DPN in NGT was only 0.4%. DPN was significantly associated with age (b: 0.098, S.E.: 0.028, OR: 1.103, CI: 1.044–1.166,  $P < 0.001$ ) and HbA1c (b: 0.523, S.E.: 0.128, OR: 1.687, CI: 1.312–2.169,  $P < 0.001$ ) by a logistic regression analysis.

**Conclusion:** Hyperglycemia was a strong risk factor for diabetic peripheral neuropathy. Peripheral neuropathy should be screened in IGR patients.

Table 1 - Multiple logistic regression analysis using DPN as a dependent variable

Variables	$\beta$	S.E. $\beta$	P	Odd ratio
Age	0.098	0.028	<0.001	1.103(1.044–1.166)
Sex	-1.429	0.482	0.003	0.239(0.093–0.616)
Waist circumference	0.041	0.02	0.045	1.042(1.001–1.084)
BMI	-0.059	0.061	0.332	0.943(0.837–1.062)
HbA1C	0.523	0.128	<0.001	1.687(1.312–2.169)
TG	0.016	0.14	0.911	1.016(0.772–1.336)
LDL	-0.154	0.297	0.604	0.857(0.479–1.535)
HDL	-1.024	0.87	0.239	0.359(0.065–1.977)
Blood pressure(mmHg)				
Systolic	-0.007	0.015	0.636	0.993(0.965–1.022)
Diastolic	-0.02	0.027	0.466	0.98(0.929–1.034)

Supported by: NSFC

## 1156

### Screening for future glucose disturbances in subjects at risk of diabetes by a rapid and non invasive measurement of sweat function

J.-H. Calvet<sup>1</sup>, G. Mueller<sup>2</sup>, J. Dupin<sup>1</sup>, P. Schwarz<sup>2</sup>;

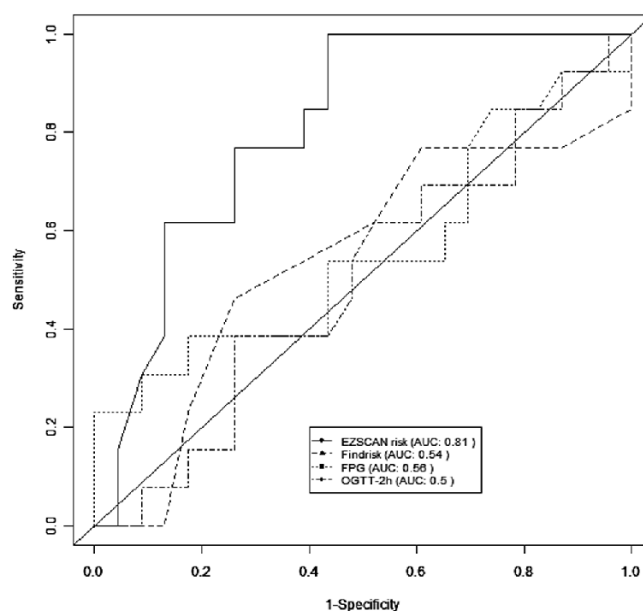
<sup>1</sup>Impeto Medical, Paris, France, <sup>2</sup>Prevention and Care of Diabetes, University of Dresden, Germany.

**Background and aims:** Several studies have shown a sweat dysfunction, witness of autonomic neuropathy, in people obese or at risk for type 2 diabetes. EZSCAN is a new quick and noninvasive technology to assess the sweat function at the palms and soles. It is based on the measurement of conductance resulting from the electrochemical reaction between the chloride ions of sweat and electrodes in contact with the skin. The purpose of this study was to compare this method with conventional measures of glucose tolerance and to evaluate its predictive for future disturbance in HbA1C in subjects with normal glucose tolerance at baseline.

**Materials and methods:** This study was conducted in 200 German subjects at risk for diabetes. The patients underwent an oral glucose tolerance test (OGTT) with measurement of the area under the curve (AUC) of glucose and insulin. Patients also completed a questionnaire to calculate their FINDRISC score. A subgroup of 76 patients received a follow up assessment after two years, among them 36 had a normal glucose tolerance at baseline. EZSCAN measurement of sweat function was performed with measurements of hands and feet electrochemical conductances (ESC) at baseline and follow-up investigation with computation of a risk score. Subjects were classified according to their sweat conductances and risk score into 3 groups: normal sweat function, moderately disturbed and highly disturbed.

**Results:** The mean age was  $56 \pm 14$  years and BMI  $28 \pm 5$  kg/m<sup>2</sup>. Depending on the values of hands and feet ESC, 43 subjects had a normal sweat function, 86 a function moderately disturbed and 71 a function highly disturbed. A significant difference was observed between the 3 groups in the AUC of glucose with the following respective values of  $836 \pm 174$ ,  $1009 \pm 202$  and  $1041 \pm 233$  mmol/Lmn,  $p < 0.0001$ . In 36 subjects with normal glucose tolerance at baseline, the AUC of the ROC curve for prediction of HbA1c  $\geq 5.7\%$ , two years later was 0.81 for the risk score EZSCAN while it was 0.54 for FINDRISC, 0.56 for fasting plasma glucose and 0.50 for 2h-plasma glucose (Figure).

**Conclusion:** This study shows that EZSCAN can be used for early detection of future glucose disturbance in subjects at risk. These results have to be confirmed on a larger population.



## 1157

### Prevalence of sudomotor dysfunction in type 2 diabetic patients attending diabetes clinics - a multicenter study

C.N. Manes<sup>1</sup>, N. Papanas<sup>2</sup>, T. Exiara<sup>1</sup>, S. Papantoniou<sup>3</sup>, E. Kirlaki<sup>4</sup>, S. Tsotoulidis<sup>5</sup>, N. Kefalogiannis<sup>4</sup>, E. Maltezos<sup>2</sup>;

<sup>1</sup>Diabetes Unit, General Hospital "Papageorgiou", Thessaloniki, <sup>2</sup>Diabetic Foot Clinic, Dimokritos University of Thrace, Alexandroupolis, <sup>3</sup>Diabetes Clinic, General Hospital of Kavala, <sup>4</sup>Diabetes Unit, Venizelion Hospital, Heraklion, Kreta, <sup>5</sup>Health Center, Kassandria, Halkidiki, Greece.

**Background and aims:** To investigate the prevalence of sudomotor dysfunction (SD) in type 2 diabetic patients on a multicenter study and assess any relation to overall nerve fibre damage.

**Materials and methods:** The study included 1010 type 2 diabetic patients randomly selected from those attending five (5) diabetes centers. There were 608 males (60.19%). Mean age and diabetes duration were  $63.90 \pm 10.26$  and  $12.24 \pm 7.75$  (yrs) respectively. A new indicator plaster method (neuropad) recently approved was used for the diagnosis of SD. Assessment of overall nerve fibre dysfunction was performed and graded clinically using the Neuropathy Disability Score (NDS). The plaster (colour blue) was applied for 10 minutes on the plantar aspect of the feet and the results recorded as pink, patchy (blue/pink), and blue. The abnormal result defined as patchy and/or blue indicated patients with SD.

**Results:** 1) 441 patients (43.7%) were found with SD - group A. They were older ( $66.74 \pm 8.78$  vs  $61.71 \pm 10.79$  yrs  $p < 0.0001$ ), had longer duration of diabetes ( $14.42 \pm 7.63$  vs  $10.54 \pm 7.42$  yrs,  $p < 0.0001$ ) in comparison with those ( $n = 569$ ) without any sign of SD - group B. 2) The signs of developed SD had 94.9% sensitivity and 70.2% specificity for overall fibre dysfunction. 3) Furthermore more severe peripheral somatic neuropathy (NDS  $6.85 \pm 5.59$  vs  $1.25 \pm 1.79$ ,  $p < 0.0001$ ) was detected in SD patients in comparison with those expressing normal SD tests. 4) In the logistic regression analysis age, diabetes duration but not sex were found to affect the development of SD ( $p < 0.05$ ).

**Conclusion:** The findings of the present study indicate that Sudomotor Dysfunction affects a large proportion of diabetic patients. The study also identifies the most important risk factors for SD, e.g. age, long diabetes duration. Proper foot care and education for this population is of great importance since SD is associated with peripheral nerve fibre damage.



## 1158

### Risk factors of impaired gastric emptying in patients with long-standing type 1 diabetes mellitus

T.T. Várkonyi<sup>1</sup>, R. Takács<sup>1</sup>, C. Lengyel<sup>1</sup>, M. Lázár<sup>2</sup>, M. Papós<sup>2</sup>, L. Pávics<sup>2</sup>, P. Kempler<sup>3</sup>, T. Wittmann<sup>1</sup>;

<sup>1</sup>1st Department of Medicine, University of Szeged, <sup>2</sup>Department of Nuclear Medicine, University of Szeged, <sup>3</sup>1st Department of Medicine, Semmelweis University, Budapest, Hungary.

**Background and aims:** Impaired gastric emptying is common among diabetic patients; however, its risk factors are poorly understood. The aim of this study was to identify potential risk factors of impaired gastric emptying among patients with long-standing type 1 diabetes mellitus.

**Materials and methods:** 54 patients with type 1 diabetes mellitus were included into the study (age: 37.1±1.74; duration of diabetes: 16.6±1.59 yrs; BMI: 24.1±0.46; HbA1c: 8.3±0.35%; mean±SE) The emptying of the stomach was evaluated by a scintigraphic gastric emptying procedure. Calculation of half-emptying time (HTE) characterized the stomach motility. Cardiovascular reflex tests were applied for the evaluation of autonomic neuropathy (AN). Three heart rate tests and two blood pressure tests were performed to assess the parasympathetic and sympathetic functions. Current perception thresholds were determined on the upper and lower limbs to study the sensory nerve integrity by a Neurometer (Neurotron Inc., Baltimore).

**Results:** The gastric emptying significantly correlated with the duration of diabetes, the age, the BMI and the HbA1c of patients (HTE-duration:  $r=0.33$ ,  $p<0.05$ ; HTE-age:  $r=0.43$ ,  $p<0.01$ ; HTE-HbA1c:  $r=0.27$ ,  $p<0.05$ ; HTE-BMI:  $r=0.33$ ,  $p<0.05$ ) A significant relationship was proven between the impaired gastric emptying and autonomic dysfunction (HTE-heart rate response to breathing:  $r=0.37$ ,  $p<0.01$ ; HTE-AN score:  $r=0.43$ ,  $p<0.01$ ). No association was found between stomach motility and sensory nerve function.

**Conclusion:** Among patients with long standing type 1 diabetes impaired gastric emptying was associated with age, diabetes duration, BMI and glycaemic control. There was a significant connection between impaired gastric motility and parasympathetic autonomic neuropathy. Effective management of risk factors, in particular, BMI and glycaemic control might contribute to the preservation of intact gastric motility in patients with type 1 diabetes mellitus.

## 1159

### Gustatory sweating - a common late diabetic complication?

T.S. Hansen, L. Tarnow;

Clinical Research Unit, Steno Diabetes Center, Gentofte, Denmark.

**Background and aims:** Gustatory sweating is a manifestation of autonomic neuropathy and is characterised by profuse sweating during or immediately after ingestion of food. It has been reported to occur more often in patients with long-standing diabetes with severe peripheral and autonomic neuropathy. Most reports on gustatory sweating have been case reports suggesting it to be a rare late diabetic complication. The aim of this study was to determine the prevalence of gustatory sweating in an unselected cohort of patients with type 1 diabetes.

**Materials and methods:** A questionnaire with 8 questions concerning gustatory sweating was designed and mailed to 745 patients with type 1 diabetes (49 % male, age: 51.5 ± 14.7 years, duration of diabetes: 26.7 ± 14.5 years, HbA1c: 8.1 ± 1.1 %) attending our outpatient clinic. The response rate was 89.3 %. Patients were classified as having gustatory sweating if they reported to suffer from sweating on the face, scalp or upper body during or immediately after ingestion of food. Patients with gustatory sweating indicated to have increased sweating as compared with others. Clinical data were extracted from patient records.

**Results:** A total of 43 (6.5 %) patients were classified as having gustatory sweating. Gustatory sweating was not associated with peripheral neuropathy, retinopathy or elevated albumin excretion rate. Patients with and without gustatory sweating were similar concerning sex, age, duration of diabetes, blood pressure and HbA1c. A trend towards a higher BMI in patients with diabetic gustatory sweating was seen (26.6 kg/m<sup>2</sup> vs 25.4 kg/m<sup>2</sup>,  $p = 0.06$ ). Patients with gustatory sweating more often also had gastroparesis: 18.6 % vs 5.2 %,  $p < 0.001$ .

**Conclusion:** Symptoms of diabetic gustatory sweating was seen in 6.5 % of patients with longstanding type 1 diabetes. Gustatory sweating was associated with symptoms of diabetic gastroparesis but not with other micro vascular complications.

## 1160

### Diabetic autonomic neuropathy in type 1 is associated with albuminuria, retinopathy, peripheral neuropathy, but in type 2 it is associated with pulse pressure and obesity

J. Fleischer<sup>1</sup>, K. Yderstraede<sup>2</sup>, E. Gulichsen<sup>3</sup>, P. Jakobsen<sup>4</sup>, H. Lervang<sup>4</sup>, E. Eldrup<sup>5</sup>, H. Nygaard<sup>6</sup>, L. Tarnow<sup>3</sup>, N. Ejlskjær<sup>1</sup>;

<sup>1</sup>Aarhus University Hospital, Department of Endocrinology and Internal Medicine, <sup>2</sup>Odense University Hospital, Department of Endocrinology, <sup>3</sup>Steno Diabetes Center, Gentofte, <sup>4</sup>Aalborg University Hospital, Department of Endocrinology, <sup>5</sup>Herlev Hospital, Department of Internal Medicine and Endocrinology, <sup>6</sup>Clinical Institute, Aarhus University, Denmark.

**Background and aims:** To identify the presence of cardiovascular autonomic neuropathy (CAN) in a cohort of individuals with diabetes in four outpatient clinics in Denmark and to evaluate the association between CAN and other risk factors.

**Materials and methods:** The DAN-Study is a Danish multi-center study focusing on diabetic autonomic neuropathy. Over a period of twelve months, 382 type 1 and 271 type 2 individuals with diabetes were tested for CAN. Patients were randomly recruited and tested during normal visits to outpatient clinics at four Danish hospitals. The presence of CAN was quantified by measuring cardiovascular reflex tests (Valsalva, response to standing and deep breathing) and analysis of heart rate variability in time and frequency domain. In order to describe possible associations, multivariate analysis with CAN as the dependent variable was performed.

**Results:** Besides heart rate above 80 ( $p<0.001$ ) and age above 60 years ( $p<0.02$ ) in both type 1 and type 2 patients multiple ordinal logistic regression analysis revealed that in type 1 diabetes patients CAN was associated with microalbuminuria ( $p=0.015$ ), macroalbuminuria ( $p=0.005$ ), and proliferative retinopathy ( $p=0.021$ ). Among type 2 diabetes patients CAN was associated with high pulse pressure ( $p<0.001$ ) and BMI above 35 kg/m<sup>2</sup> ( $p=0.025$ ).

**Conclusion:** In this cross-sectional observational study CAN is associated with proliferative retinopathy, micro- and macroalbuminuria in type 1 diabetes patients, whereas in type 2 diabetes patients CAN is associated with pulse pressure and obesity.

## 1161

### High prevalence of unawareness of having distal sensorimotor polyneuropathy in diabetes and pre-diabetes; the KORA F4 Study

B. Bongaerts<sup>1</sup>, W. Rathmann<sup>1</sup>, M. Heier<sup>2</sup>, B. Kowall<sup>1</sup>, D. Stöckl<sup>2</sup>,

C. Meisinger<sup>2</sup>, D. Ziegler<sup>3,4</sup>;

<sup>1</sup>Institute of Biometrics and Epidemiology, German Diabetes Center, Düsseldorf, <sup>2</sup>Institute of Epidemiology II, Helmholtz Zentrum München, Neuherberg, <sup>3</sup>Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf, <sup>4</sup>Department of Metabolic Diseases, Düsseldorf University Hospital, Germany.

**Background and aims:** Distal sensorimotor polyneuropathy (DSPN) is a severe complication of type 2 diabetes. Whereas its prevalence is extensively studied, data on undiagnosed DSPN are sparse. The current study aimed to assess the prevalence of unawareness of having DSPN in pre-diabetes and diabetes, using a sample of the older population of Augsburg, South-Germany.

**Materials and methods:** Glucose tolerance status was determined in 61-82 year-old participants of the population-based KORA F4 Study (2006-2008) ( $n=1,100$ ). Clinical DSPN was defined as the presence of bilaterally impaired foot-vibration perception and/or bilaterally impaired foot-pressure sensation. DSPN cases were considered unaware of their condition when answering "no" to the question "has a physician had ever told you that you are suffering from nerve damage, neuropathy, polyneuropathy, or diabetic foot?"

**Results:** Clinical DSPN was prevalent in 154 (14%) participants, 140 of whom were unaware of their disorder (table). With 11 out of 46 subjects (23.9%, 95%CI: 12.6-38.8), participants with combined impaired fasting glucose and impaired glucose tolerance (IFG-IGT) had the highest prevalence of DSPN. Of these, 10 (91%) were unaware of having clinical DSPN. Participants with known diabetes had an equally high prevalence of clinical DSPN (22.0%, 95%CI: 16.2-28.9), with 30 out of the 39 (77%) cases with DSPN being unaware of having the disorder. Information on the performance of foot examinations was available for participants with known diabetes only ( $n=167$ ). In total of 113 (68%) subjects with known diabetes reported to have had their feet examined by a physician, whereas about a quarter reported to have never undergone a foot examination. A total of 38 out of 167 participants with known diabetes had clinical DSPN, 29 (76%) of whom were unaware of having the disorder.

**Conclusion:** Our findings show a high prevalence of unawareness of having clinical DSPN among subjects with IFG-IGT and with diabetes, and an insufficient frequency of foot examinations by a physician, suggesting an inadequate attention to diabetic foot prevention practice.

**Table:** Prevalence of clinical distal sensorimotor polyneuropathy, according to glucose tolerance status; KORA F4 (2006–2008)

	n	Prevalence clinical DSPN <sup>1</sup> (95% CI)	Unawareness of clinical DSPN <sup>2</sup>
Total study population (n=1,100)	154	14.0% (12.0–16.2)	91%
Normal glucose tolerance (n=577)	64	11.1% (8.6–13.9)	98%
Isolated-impaired fasting glucose (n=55)	3	5.5% (1.1–15.1)	100%
Isolated-impaired fasting glucose (n=183)	27	14.8% (10.0–20.7)	89%
Impaired fasting glucose - impaired glucose tolerance (n=46)	11	23.9% (12.6–38.8)	91%
Newly diagnosed diabetes (n=62)	10	16.1% (8.0–27.7)	100%
Known diabetes (n=177)	39	22.0% (16.2–28.9)	77%

CI: confidence interval, DSPN: distal sensorimotor polyneuropathy

<sup>1</sup> Defined as the presence of an impaired bilateral foot-vibration perception and/or an impaired bilateral foot-pressure sensation.

<sup>2</sup> Defined by a disaffirmative answer to the question “has a physician ever told you that you have nerve damage, neuropathy, polyneuropathy or diabetic foot?” in combination with the presence of clinical DSPN.

Supported by: German Research Foundation

## 1162

### Small nerve fibre dysfunction is more pronounced than large fibre involvement in type 1 diabetes of forty years duration

K.A. Sveen<sup>1</sup>, B. Karime<sup>2</sup>, E. Jørum<sup>3</sup>, K. Dahl-Jørgensen<sup>3</sup>, S.I. Mellgren<sup>4</sup>, K.F. Hanssen<sup>1</sup>;

<sup>1</sup>Dept of Endocrinology, Oslo University Hospital, <sup>2</sup>Section of Clinical Neurophysiology, Oslo University Hospital, Rikshospitalet, <sup>3</sup>Dept of Pediatrics, Oslo University Hospital, Norway, <sup>4</sup>Dept of Clinical Medicine (Neurology), University of Tromsø, Norway.

**Background and aims:** Diabetic neuropathy is a major health problem that can lead to foot ulceration and lower limb amputation. Small fibers constitute 70–90% of peripheral nerve fibers and are suggested to be the earliest fibers to be damaged in diabetes. The prevalence of small fiber neuropathy and the mechanisms of its development are however not known in detail in long term type 1 diabetes. We therefore evaluated large and small nerve fiber function in a well characterized cohort with a long duration of type 1 diabetes by analyzing thermal thresholds and intraepidermal nerve fiber density (IENFD) in the skin, HbA1c and the advanced glycation endproduct Nepsilon-(carboxymethyl) lysine (CML) in serum.

**Materials and methods:** Twentyseven persons (53±7 years) with type 1 diabetes of 40±3 years duration from the “Oslo study cohort” underwent neurophysiological investigations of large- and small nerve fibers. Symptom evaluation and neurological examination were performed. Large fiber function was assessed by nerve conduction studies (NCS), small fibers by quantitative sensory thresholds (QST) and IENFD from the distal leg for quantification of small nerve fibers. HbA1c was measured prospectively for 27 years. The advanced glycation endproduct CML (Carboxymethyllysine) was measured ten years prior to these examinations by immunoassay.

**Results:** Only 30% reported sensory symptoms. NCS were normal in 11 (41%) patients. The remaining 16 displayed abnormal findings compatible with neuropathy, slight in four, moderate in eight and severe in four patients. All patients with symptoms had abnormalities on NCS. Thermal thresholds in the feet and at the level of the knee were elevated in the patients compared to healthy controls. Twentytwo (81%) patients were diagnosed with small fiber dysfunction (cold detection <25 °C on the dorsum of the feet and/or warm

detection >43 °C), either a pure small fiber neuropathy (with normal NCS) in 7 patients or a small fiber dysfunction in addition to large fiber neuropathy (n=15). IENFD was abnormal in 19 (70%) and significantly lower in diabetic patients than in age matched controls (4.3 mm±2.3 vs 11.2 mm±3.5, p<0.001). IENFDs were negatively correlated to warm threshold temperature in the leg (r=-0.56, p=0.002), knee (r=-0.52, p=0.005) and heat-pain threshold in the leg (r=-0.57, p=0.002). Mean HbA1c over 27 years correlated negatively with IENFD (r=-0.4, p=0.043), heat-pain threshold in the hand (r=-0.5, p=0.02) and to cold detection threshold (r=-0.6, p=0.002). In a multivariate linear regression model including age, height, BMI and CML, all were significantly associated with low IENFD (adjusted R<sup>2</sup>=0.57).

**Conclusion:** Small fiber sensory neuropathy presenting with reduced IENFD densities and correlated warm thresholds evaluated by QST is a major manifestation in type 1 diabetes of forty years duration, and is more pronounced than large fiber neuropathy evaluated clinically and by nerve conduction. HbA1c and the advanced glycation endproduct CML seem to be important factors in the development of small fiber damage in long term type 1 diabetes. Our findings may indicate that CML has a predictive value in the development of small fiber dysfunction in type 1 diabetes.

Supported by: The South-Eastern Norway Regional Health Authority

## 1163

### A 16-year prospective study of autonomic nerve function in type 1 diabetic patients: association with autoimmunity to nervous tissue structures

E. Favaro<sup>1</sup>, A. Raviolo<sup>1</sup>, E. Coppo<sup>1</sup>, K. Bonomo<sup>2</sup>, P. Passera<sup>1</sup>, P. Massucco<sup>2</sup>, A. Blatto<sup>3</sup>, A. Grassi<sup>4</sup>, G. Grassi<sup>1</sup>, M. Porta<sup>1</sup>, P. Cavallo Perin<sup>1</sup>, R. Quadri<sup>1</sup>, M.M. Zanone<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, University of Turin, <sup>2</sup>Ospedale San Luigi Gonzaga, University of Turin, Orbassano, <sup>3</sup>Ospedale Maria Vittoria, Divisione di Diabetologia, <sup>4</sup>Ospedale Mauriziano, Divisione di Diabetologia, Turin, Italy.

**Background and aims:** Data on the early stage and the natural history of autonomic and somatic neuropathy in type 1 diabetes are scanty. Studies have linked autoimmunity to autonomic nervous tissue structures and autonomic neuropathy. To evaluate prospectively this association, we monitored autonomic function and the presence of autoantibodies (Ab) to sympathetic and parasympathetic nervous structured in a cohort of young type 1 diabetic patients.

**Materials and methods:** One hundred and twelve young type 1 diabetic patients (age >11 years, disease duration > 3 years) were recruited between 1995–1997 (time 1). Anti-vagus nerve and anti-cervical ganglia Ab were detected in 28 patients (14 patients had anti-vagus Ab, 14 had anti-cervical ganglia Ab, 1 had both Ab), by indirect IF complement-fixation technique. 92 (82%) patients were re-evaluated at time 2 (40 ± 3 months from time 1), and 57 (51%, -of whom 48 completed all tests) were re-evaluated at time 3 (16.6 ± 5 years from time 1) (age 31 ± 2 years, disease duration 23 ± 4 years). A standard direct enquiry for autonomic and peripheral sensory-motor symptoms was made; neurological function was assessed by performing four cardiovascular (CV) tests (deep breathing test, DB; 30/15 ratio; Valsalva ratio, VR; blood pressure drop on standing, BP), clinical and neurological examination, vibratory perception threshold (VT) measurement.

**Results:** At time 3 follow-up, CV test values were significantly lower compared to time 1 and time 2 values. Dividing the patients according to the nervous tissue Ab status, one test was borderline in 6 (6/34, 17%) Ab- patients, and one or more tests abnormal in 9 (9/14, 64%) Ab+ patients [1 test (4 pz), 2 tests (3 pz) o 3 tests (2 pz)] (p<0.05), and time 3 CV test values were lower amongst the Ab+ compared to Ab- patients (p=0.05 only for DB test). Amongst the Ab+ patients, one patient reported gustatory sweating and one had BP drop > 20 mmHg. VT was significantly higher amongst patients with depressed reflexes (n=15), and 6 patients (4 with depressed reflexes) developed symptoms of peripheral somatic neuropathy (3 patients with carpal tunnel). There was no association between neurological parameters, presence of microvascular complications and metabolic control. Mean HbA1c value of the past 1 year (7.7% ± 1.3) significantly correlated with the mean of the 5 past years (7.8 ± 1).

**Conclusion:** The preliminary results of the ongoing prospective study of a cohort of type 1 diabetic patients indicate that symptomatic autonomic neuropathy is not characteristic of young diabetic patients, but autoantibodies to autonomic structures are associated with subtle autonomic dysfunction.

## PS 101 Foot ulceration and amputation: treatment outcomes

1164

**Time of healing of foot ulcers among patients with type 1 and type 2 diabetes have decreased in the period 2001 - 2010**

A. Nielsen, T.P. Almdal, K.E. Nielsen, U. Bjerre-Christensen, A. Rasmussen, P. Holstein;

Steno Diabetes Center, Gentofte, Denmark.

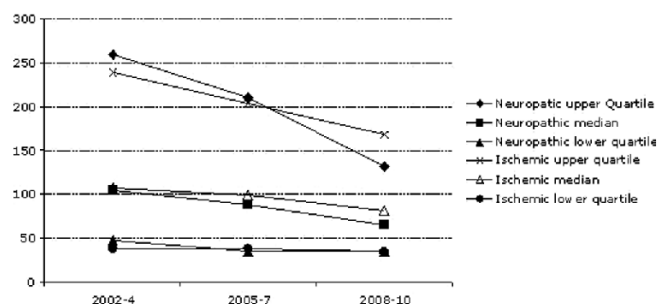
**Background and aim:** Foot ulcers are a very costly complication among diabetes patients. If the time from diagnosis to healing of the ulcer can be reduced this will decrease the costs. To our knowledge there are no longitudinal studies of change in healing time of foot ulcers among diabetes patients. The aim of the present study was to study the possible changes in healing of foot ulcers in two large cohort of type 1 diabetes patients (T1D) and type 2 diabetes patients (T2D) in the period 2002-2010.

**Methods:** Our clinic is a specialized diabetes clinic. The clinic has a multidisciplinary foot care team and is an integrated part of the public health care system in the greater Copenhagen area. In the clinic app 3500 patients with T1D and app 2000 patients with complicated T2D are followed. When a foot ulcer is observed the patients is seen immediately by the podiatrist and if necessary a specialist in orthopedic surgery within at max 1 week. At the initial visit it is determined whether etiology of the ulcer is neuropathic or ischemic. Standard treatment consist of therapeutic footwear with individual off-loading, antibiotics if infection, surgical drainage when required, referral to corrective surgery or vascular surgery if indicated, and education. Patients are seen with 1-6 weeks interval until healing of the ulcer. All information in relation to the patients, including date of diagnosis as well as date of complete healing of ulcers is housed in an electronic patient record system. We studied the healing time of foot ulcers, the number of visits until healing, the number of days without an ulcer before a new ulcer occurred, and the development in these figures over a nine years period.

**Results:** In the 2002-04 a total of 548 ulcers (60% toe ulcers) were diagnosed, in 2005-07 a total of 627 ulcers (55% toe ulcers) were diagnosed, and in the period 2008-10 a total of 654 (57% toe ulcers) were diagnosed. 62 % of the ulcers were diagnosed among T2D patients and 38 % among T1D patients. For all foot ulcers healing time decreased from 104 in 2002-04 to 83 days in 2008-10. For T1D patients healing time decreased from 111 to 84 days in the period. For T2D patients healing time decreased from 99 to 82 days. The figure shows change in treatment time in relation to toe ulcers. In 2002-04 patient were seen 5 times (median) in the foot clinic before healing of the ulcer, in 2008-10 this was reduced to 3 times (median). Among patients where ulcers healed in 2002 45 % were free of any new diabetic ulcer in the subsequent 3 years, among patients where ulcers healed in 2008 55 % were free of any ulcers in the subsequent 3 years.

**Conclusion:** The present study shows that healing time of ulcers treated in a specialized diabetes clinic have decreased substantially in both patients with T1D and T2D, however most pronounced among patients with T1D.

**Days of treatment ulcers on toes, median, upper and lower quartile**



1165

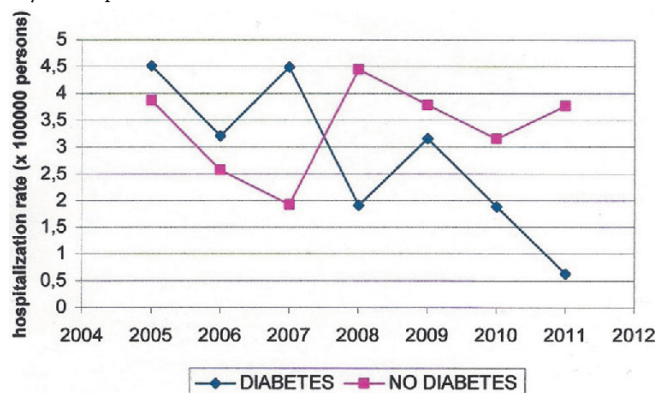
**Reduction of major amputations in diabetics after the introduction of a multidisciplinary team for the diagnosis and treatment of diabetic foot**  
S. Acquati, L. Gagliardi, A. Tartaglia, L. Buci, S. Taroni, E. Gozzo, M. Nizzoli;  
Endocrinology and metabolic disease Unit, Forlì, Italy.

**Background and aims:** Lower extremity amputation (LEA) is one of the most feared diabetic complications. 15-25% of diabetics will develop a foot lesion during their life. Despite of a precocious and intensive treatment these lesions can take weeks or months to heal. This can determine a physical inability and especially a reduction in quality of life. A lesion can also often precedes a major limb amputation. Several studies show how a prompt and precocious treatment by a multidisciplinary team is able to reduce the risk of LEA. Since 2007 in Forlì area (Northern Italy) diabetic foot was managed by different specialist in a chaotic way. In September 2007 it was created a multidisciplinary team for the diagnosis and treatment of diabetic foot coordinated by an endocrinologist, expert in this field. LEA is considered a marker of the quality of foot care in diabetics. Aim of the study was to verify the efficacy of the introduction of a multidisciplinary diabetic foot approach in our hospital evaluating the trend of amputation rate from 2005 to 2011.

**Materials and methods:** All major LEAs from 1 January 2005 to 31 December 2011 were identified. We used hospital discharge data to identify patients. We selected the procedure codes 8415 and 8417 (non traumatic major amputation) from 2005 until 2011. Then we analyzed the principal and/or secondary diagnosis of diabetes (ICD-9 codes 250) and we divided the patients who underwent a major amputation in two group: group D (patients affected by diabetes) and group ND (patients not affected by diabetes). We also used as data source diabetes register of Forlì. We evaluated the number of major LEAs from 2005 to 2011, the age of patients and duration of hospitalization (days) in both groups.

**Results:** From 2005 until 2011 we registered n° 68 major limb amputation in Forlì (D 31/68% - 45,5% vs ND 37/68 - 54,4 %). At the 2005 census Forlì had a population of 155503, predominantly white caucasian; at 2006 population was 155612; at 2007 it was 155728; at 2008 it increased to 157261; at 2009 population was 158417; at 2010 it was 158823 and 158913 at 2011. We observed a reduction in major amputation hospitalization rate from 2005 until 2011 in diabetic population compared to general population (see graph below). The analysis of clinical data showed an older age in general population (D 75.6±7.3 yrs vs ND 82.9±3.9 yrs, p=0.038). We didn't see a significant difference in days of hospitalization between the two group (D 29.4±19.4 days vs ND 41.6±27.5 days, p=n.s.).

**Conclusion:** This study shows that a multidisciplinary approach for the diagnosis and treatment of diabetic foot, structured and coordinated by a specialist, reduce the major LEAs rate in diabetics and seems to slightly reduce the days of hospitalization.



1166

**Strategy to improve diabetic foot care: results of 7-year prospective study from implementation to global approach**

R. Anichini<sup>1</sup>, A. Tedeschi<sup>1</sup>, S. Baroncelli<sup>1</sup>, A. Bernini<sup>1</sup>, L. Barbanera<sup>1</sup>, G. Seghieri<sup>1</sup>, M. Marini<sup>2</sup>, R. Salvadori<sup>2</sup>, A. De Bellis<sup>1</sup>;

<sup>1</sup>Internal Medicine, Diabetes Unit, <sup>2</sup>Società Salute, Public Health Area, Pistoia, Italy.

**Background and aims:** In 2007 we published the results of a 5-year period study (1999-2003), performed to evaluate whether the implementation of a



programme for prevention and treatment of diabetic foot lesions could decrease the number of amputations in the area of our interest (Pistoia, 171,000 inhabitants). Following the establishment of a strategy including prevention, patient and staff education, multidisciplinary treatment of foot ulcers and close monitoring, diabetic foot care resulted greatly improved in terms of lower extremity amputations and final outcome of the lesions. The implementation of the International Consensus on Diabetic foot lesions in Pistoia was introduced in Pistoia at level of Primary Care (Health Care District, general practitioners), and at level of Secondary Health Care through the creation of a multidisciplinary care team at the hospital, including the diabetologist, specialized nurses, podiatrist, orthopaedic surgeon and cardiologist. Recently, the collaboration with GPs has been further reinforced through the development of the Chronic Care Model (CCM) which ensures to patients an integrated assistance between GPs and specialists (2009). The aim of the present study was to evaluate:

1. the increasing percentage of patients referred to our unit for diabetic foot lesions, who underwent a structured educational programme including footwear during years 2004–2011,
2. the possible reduction of the number of severe foot lesions and of amputations occurring during these years,
3. the increasing number of revascularization procedures performed in the same period of time.

**Materials and methods:** Clinical records are kept by the diabetologist and the database for this study was taken from DRG Tuscany database, from Eurotouch® and CCM database

**Results:** The data of this study demonstrate:

1. a 40% increase of patients referred for the first time to our unit (from 496 in 2004 to 806 in 2011) who underwent the primary prevention educational programme including foot care,
2. a significant reduction of numbers of lesions seen (45%) (from 340/years in 2004 to 186 ys in 2011) and reduction lesions with a high grade (WTCS) of severity (>2) (20%) in outpatients of the observation of severe foot lesions (gangrene and serious infections)
3. a reduction of incidence of major amputations (from 3 x 100000 inhabitants in 2004, to 1.8 in 2011;  $p < 0.01$ ), while a different trend was observed concerning minor amputations, with an incidence rate of 3,5 in 2004 and 6,2 in 2011.
4. following the increasing collaboration with the cardiologist dedicated to revascularization procedures, an increasing number of below knee endovascular procedures (BKEP) has been performed (from 10 in 2004 to 120 in 2011).
5. in these periods outpatients treated by podiatrist in primary and secondary prevention were increased from 160 to 922.

**Conclusion:** Analysis of these preliminary data show that, following the implementation of ICDF on Diabetic Foot a significant improvement of foot care in diabetic patients has been observed, and in the period of our observation the percentage of the patients seen by the foot care team has become progressively higher, such that now in the health area of our interest all diabetic patients are screened for diabetic foot every years and patients with high risk of ulceration or with diabetic foot are referred to the local Diabetic foot unit.

## 1167

### Therapeutic footwear of 54 patients with Charcot diabetic foot

G. Ha Van;

Diabetologia PR Hartemann, Hôpital Pitié - Salpêtrière, Paris, France.

**Background and aims:** After healing a plantar ulcer on deformation of a Charcot foot we studied the recurrence of the ulcer after therapeutic footwear. The objective was to study the avoidance of surgical correction of deformation of the Charcot foot by made to measure shoes.

**Materials and methods:** We followed prospectively 54 diabetic patients with Charcot foot. 46/54 patients had a history of ulcer which was healed by off loading. Every patient (n=54) has got a therapeutic footwear made in our team by the same shoe-maker. We studied the recurrence or the occurrence of plantar ulcers and the average duration of wearing of the footwear.

**Results:** 5% of the 54 patients had a history of plantar ulcer on the Charcot foot deformation 100% of ulcers healed (average duration of ulcer: 241 days). The average data were: age: 58 years diabetes age: 18 years, type 2 diabetes : 98%, BMI: 28.6, Right foot: n=20 Left Foot n= 20, 10 Bilateral Charcot foot, 92% had a midfoot localisation. Average duration of follow up of the wearing of therapeutic shoes:  $882 \pm 899$  days. Average duration of daily use of the shoes:  $7 \pm 5$  hours per day  $2 \pm 2$  hours of walking, average distance of walking without stopping:  $1559 \text{ m} \pm 2324$ . Amputation: below-knee: 3%, toe:

3%, death: 3%. Rate of recurrence of ulcer on the deformation of the Charcot Foot = 9%.

**Conclusion:** Corrective surgery of the Charcot foot (92% of midfoot localisation) has been avoided by wearing therapeutic footwear made by our specialised team: 91% of absence of plantar ulcers after 882 days of follow-up.

## 1168

### Eight year outcomes of a consecutive single centre series of patients presenting with osteomyelitis of the foot in diabetes

W.J. Jeffcoate, F.L. Game;

Diabetes and Endocrinology, Nottingham University Hospitals Trust, UK.

**Background and aims:** To determine the outcome at 5 and 8 years of patients newly diagnosed with osteomyelitis of the foot in diabetes.

**Materials and methods:** Observational study of a consecutive series managed at a single centre.

**Results:** The baseline data of this series of 147 patients has been previously published, together with outcomes at 12 months. Mean age was 64.7 years, 66% men. There was an apparent resolution of bone infection in 63.3% cases, and 23% of the population had had a minor amputation and 8.8% had had a major amputation by 12 months. Full 8 year outcome data are available in 138 (93.9%) of the 147 patients. By 5 years a total of 37 (26.8%) had had minor amputations on the side of the original lesion, and 12 (8.6%) had had a minor amputation on the other side. 18 (12.9%) had had a major amputation on the ipsilateral side and 6 (4.3%) had had one on the contralateral side, including one with bilateral limb loss. 71 (51%) of the series had died within 5 years. By 8 years there had been a further 3 minor amputations on the index limb (total 8 year ipsilateral minor amputations, 28.9%), a further 6 contralateral minor amputations (total 13%). There had been 2 further major amputations (1 ipsilateral and 1 contralateral), leaving a total of 17.7% who had lost one or both limbs by this time. 61.9% of the cohort had died by 8 years, in a median of 33 (range 1–96) months post presentation. In those who had major amputation the median time to death was 31 (range 1–78) months post amputation.

**Conclusion:** The long term outcome for patients newly diagnosed with osteomyelitis of the foot in diabetes is poor with over half dying before 5 years of follow-up. The poor prognosis is factor that should be taken into account when considering the options for long-term management. Despite this, there is no evidence that the relatively conservative approach to the management of this series resulted in a worse outcome than any previously reported.

## 1169

### Neurotensin and collagen dressings improve diabetic wound healing

L. Moura<sup>1,2</sup>, A. Dias<sup>2</sup>, E. Suesca<sup>3</sup>, S. Casadiegos<sup>3</sup>, M.F. Duque<sup>3</sup>, H. Sousa<sup>2</sup>, E. Carvalho<sup>1,4</sup>;

<sup>1</sup>Center for Neuroscience and Cell Biology, Coimbra, Portugal, <sup>2</sup>CIEPQPE, Chemical Engineering Department, University of Coimbra, Portugal,

<sup>3</sup>Pharmacy Department, Universidad Nacional de Colombia, Bogotá,

Colombia, <sup>4</sup>APDP, The Portuguese Diabetes Association, Lisboa, Portugal.

**Background and aims:** Impaired wound healing is an important clinical problem in diabetes and results in failure to completely heal diabetic foot ulcers (DFU), leading to lower extremity amputations. Recent studies indicated that neuropeptides like Substance P, NPY, and CRH may act as inflammatory modulators and may improve the diabetic wound healing process. However, to enhance the DFU wound healing process, wounds should be dressed with appropriate biomaterials to protect and avoid contaminations and to provide a sustained and effective release of bioactive substances. Collagen and chitosan biopolymers have been used for this purpose due to its favorable properties such as biocompatibility, biodegradability, non-toxicity and favorable biological behavior. The aim of this work is to use a wound dressing system for neurotensin delivery, into the wound, using collagen as biopolymer dressing.

**Materials and methods:** Animal model: Diabetes was induced by an intraperitoneal injection of 200mg/kg streptozotocin (STZ) dissolved in 200ml citrate buffer (pH 4.2) or buffer alone (non-diabetic mice). Control or STZ-treated mice were anesthetized and two 6 mm excision wounds, 2 cm apart, were created dorsally using a punch biopsy tool. A film of collagen alone, NT alone (50ug/wound/per day), collagen loaded with NT (50ug/wound/per day) or PBS were placed daily on wounds till total healing and the progress of wound closure was monitored by acetate tracing up to 10 days. Tissue analysis: Gene expression of inflammatory factors (TNF-alpha, IL-6) and

MMP-9 involved in the wound healing pathways were measured by real-time RT-PCR.

**Results:** In diabetic mice, collagen treated wounds showed a reduction in the wound area as compared to PBS treated wounds (13%), NT treatment alone showed a reduction of (14%), while it is with the combination of the two treatments that we observed the greatest reduction in wound area (19%,  $p < 0.01$ ), effects were significant already at 3 days post-wounding. A major expression of inflammatory factors is observed at day 3 compared to day 0 in non-treated wounds. At day 3, both TNF- $\alpha$  and IL-6 are up regulated in controls and diabetic mice while this effect decreased back to day 0 levels in non-treated wounds. Similar results are observed for MMP-9. Collagen alone significantly decreased TNF- $\alpha$  ( $p < 0.05$ ) and MMP-9 ( $p < 0.05$ ) gene expression in diabetic compared with control mice at day 3. At day 10 post-wounding, similar results were observed. In addition, at day 3, collagen in combination with NT decreased the highly expressed TNF- $\alpha$  and MMP-9 observed with NT treatment in diabetic mice. Under these conditions, NT alone, at day 3, seems to induce TNF- $\alpha$  and MMP-9 expression and collagen treatment decreased this effect.

**Conclusion:** Results demonstrate that collagen alone or in combination with NT potentially decrease the inflammatory conditions observed in the wound at day 3, making it a potentially advantageous wound dressing for the treatment of diabetic foot ulcers.

Supported by: FCT (PTDC/SAU-MII/098567/2008, PTDC/SAU-BEB/71395/2006, SRFRH/BD/60837/2009)

## 1170

### Intravenous antimicrobial therapy for patients with diabetic foot ulcers: an effective outpatient program in Tanzania

Z.G. Abbas<sup>1,2</sup>, J.K. Lutale<sup>1</sup>, L.K. Archibald<sup>3</sup>;

<sup>1</sup>Internal Medicine, Muhimbili University of Health and Allied Sciences,

<sup>2</sup>Abbas Medical Centre, Dar es Salaam, United Republic of Tanzania,

<sup>3</sup>Infectious Diseases, College of Medicine, University of Florida, Gainesville, USA.

**Background and aims:** Foot ulcers cause substantial morbidity and mortality among persons with diabetes in Tanzania, and are associated with prolonged hospital stays and increased hospital costs. Because of these costs, and limited inpatient services and available beds, we initiated a program for instituting intravenous (IV) antimicrobial therapy in the diabetes outpatient clinics at two major centers in Dar es Salaam, Tanzania.

**Materials and methods:** During February 2005–December 2011 (study period), all adult patients with diabetes who presented at diabetic clinic at two diabetes centres in Dar es Salaam, Tanzania with infected foot ulcers were evaluated then started on courses of IV antimicrobials for 10–20 days. Patients were followed up daily by a trained nurse assistant for daily IV antimicrobials, ulcer assessments, and dressing changes at the two centres outpatient clinics. Antimicrobial therapy included various combinations of second and third-generation cephalosporins, penicillins, macrolides, quinolones, and anti-anaerobic agents.

**Results:** Logistic regression analysis was carried out and adjusted odds ratio (AOR) and 95% confidence intervals (CI) were calculated. Of 909 patients who were treated during the study period, 595 (65%) were male; median age and duration of diabetes was 55 years and 8 years, respectively. Nine hundred patients received an extended spectrum third-generation cephalosporin; complete ulcer healing was attained in 760 (84%). Independent factors associated with poor ulcer healing included macrovascular disease (AOR: 2.1, CI: 1.4–3.2); hypertension (AOR: 1.6, CI: 1.04–2.4); and concomitant administration of amoxicillin/clavulanic acid (AOR: 2.7, CI: 1.5–4.7) or quinolones (AOR: 5.1, CI: 3.3–7.9). Independent factors associated with complete ulcer healing included palpable peripheral foot pulses (AOR: 3.1, CI: 2.1–4.5), ulcers of area  $< 1,000 \text{ mm}^2$  (AOR: 2.1, CI: 1.4–3.1), or concomitant receipt of an anti-anaerobic agent (AOR: 4.5, CI: 3.0–6.8). No deaths or side effects were documented.

**Conclusion:** We established the feasibility of affordable, outpatient IV antimicrobial therapy in the management of infected diabetic foot ulcers in Tanzania. Complete healing was realized in 84% of patients with best outcomes documented among patients who received a single extended spectrum third-generation cephalosporin along with an anti-anaerobic agent. Addition of other antimicrobial agents, including penicillins, macrolides, or quinolones, did not enhance ulcer healing rates. These findings potentially have cost saving implications for the management of infected diabetic foot ulcers in Tanzania.

## 1171

### Randomised, double-blind versus placebo, proof of concept clinical trial to evaluate efficacy and safety of g.68.γ/etoh in diabetic infected foot ulcers (DANTE study)

M. Monami<sup>1</sup>, S. Genovese<sup>2</sup>, R. Anichini<sup>3</sup>, C. Fondelli<sup>4</sup>, F. Romagnoli<sup>5</sup>, N. Bartoli<sup>1</sup>, G. Clerici<sup>2</sup>, G. Navales<sup>6</sup>, E. Mannucci<sup>1</sup>, G. Gensini<sup>1</sup>;

<sup>1</sup>AOU Careggi, Florence, <sup>2</sup>IRCCS Multimedica, Milan, <sup>3</sup>Azienda USL 3,

Pistoia, <sup>4</sup>Policlinico Le Scotte, Siena, <sup>5</sup>INRCA, Ancona, <sup>6</sup>L. Molteni & C.,

Florence, Italy.

**Background and aims:** RLP068/Cl is a photosensitizer compound coming from an original Italian research that showed pharmacological activity in reducing the bacterial load in vitro and in vivo models. The activity of RLP068/Cl is due to the production of singlet oxygen or other reactive oxygen species (ROS), generated through its activation by red light released by laser machine. The efficacy of activated RLP068/Cl was evaluated in vitro and in vivo models against a wide range of bacteria and yeast. No evidence of bacterial resistance was seen. RLP068/Cl (formulated into a gel formulation G.68.γ/EtOH) was found to be well tolerated in a previous trial. The object of the present trial was to demonstrate the proof of concept of the activity and tolerability of RLP068/Cl (into gel G.68.γ/EtOH) in reducing the bacterial load in infected diabetic foot ulcers.

**Materials and methods:** 62 diabetic patients with infected foot ulcers were randomized to placebo or to G.68.γ/EtOH gel (0.1%, 0.3% o 0.5%). After a mild sharp debridement in the peripheral area, gel was applied on the ulcer and after 1 hour from the application, each ulcer was illuminated with red light (689 nm) for 500 sec. The primary endpoint was the reduction of bacterial load between pre-treatment and immediately after illumination as measured by an ulcer sample drawn with a flocked nylon swab. Starting from post-illumination swab collection, all patients received an antibiotic treatment (amoxicillin trihydrate/clavulanic potassium) and they were followed for two weeks.

**Results:** Immediately after the photoactivation of the gel, total bacterial load was reduced with the active treatment 0.3% and 0.5% in respect to placebo (placebo,  $m \pm SD$  from  $4.7 \pm 0.9$  to  $3.7 \pm 1.7$  LogCFU/ml; G.68.γ/EtOH 0.1%: from  $4.4 \pm 0.8$  to  $2.5 \pm 2.0$  LogCFU/ml; G.68.γ/EtOH 0.3%: from  $4.8 \pm 0.8$  to  $1.9 \pm 1.9$  LogCFU/ml; G.68.γ/EtOH 0.5%: from  $4.4 \pm 1.0$  to  $1.4 \pm 1.7$  LogCFU/ml) with a statistical significance ( $p < 0.001$ ). This effect is still present but less evident after 3 days. Moreover the two treatments (0.3% and 0.5%) resulted in a reduction of about 3 log respect to pre-treatment. No serious adverse events related to the study drug were recorded.

**Conclusion:** The results of the study showed that RLP068/Cl is able to reduce the bacterial load in infected diabetic foot ulcers. This effect, demonstrated for the first time in patients, strongly confirms the pre-clinical data and supports the development of RLP068/Cl as an innovative treatment for infected diabetic foot ulcer.

Clinical Trial Registration Number: EudraCT Number 2010-019598-13

Supported by: L. Molteni & Co

## 1172

### Topical allogeneic mesenchymal stem cell therapy for diabetic ulceration

A. O'Loughlin<sup>1</sup>, M. Kulkarni<sup>2</sup>, M. Creane<sup>1</sup>, E.E. Vaughan<sup>1</sup>, E. Mooney<sup>1</sup>, G. Shaw<sup>1</sup>, M. Murphy<sup>1</sup>, P. Dockery<sup>3</sup>, A. Pandit<sup>2</sup>, T. O'Brien<sup>1</sup>;

<sup>1</sup>Regenerative Medicine Institute, <sup>2</sup>Network of Excellence for Functional

Biomaterials, <sup>3</sup>Department of Anatomy, National University of Ireland,

Galway, Ireland.

**Background and aims:** Non-healing diabetic foot ulceration results in a significant burden on individual patients' health and health-care system resources. Typically applied mesenchymal stem cells (MSCs) provide a new treatment approach for reducing time to wound healing. MSCs are known to promote angiogenesis. We tested the hypothesis that topically applied allogeneic MSCs increase wound healing and support angiogenesis.

**Materials and methods:** A topical cell based therapy was developed by seeding allogeneic non-diabetic bone-marrow derived MSCs in a type 1 collagen scaffold. The cells were delivered to a full thickness cutaneous wound in the alloxan-induced diabetic rabbit ear ulcer model at increasing doses. The animals remained hyperglycaemic for the duration of the study (5 weeks). The experiments were performed under license from the Department of Health with ethical approval from the National University of Ireland, Galway ethics committee. The groups analysed included untreated wounds and wounds treated with either collagen scaffold alone, collagen seeded with 50,000,

100,000 or 1,000,000 MSCs. 5 wounds were analysed per animal. Percentage wound closure after 1 week was assessed using wound tracings. Stereology was used to assess the new vascular bed in the wounds.

**Results:** 1,000,000 MSCs demonstrated significantly increased percentage wound closure in comparison to untreated wounds. Analysis between groups was by ANOVA and post-hoc Fisher's test. Statistical significance was taken as  $p < 0.05$ . Collagen and MSC-seeded collagen scaffolds demonstrated increased surface density of blood vessels and decreased radial diffusion distance when compared to controls. New blood vessels were longer had more convoluted in wounds treated with collagen seeded with 1,000,000 MSCs.

**Conclusion:** Allogeneic non-diabetic MSCs seeded in a collagen scaffold demonstrate superior cutaneous wound healing in a pre-clinical model of diabetic ulceration. Collagen and collagen seeded with MSC treatments result in increased angiogenesis when compared to untreated wounds but statistically significant increased wound healing was observed only at the higher dose of 1,000,000 MSCs. This MSC therapy delivered using a collagen scaffold leads to augmented wound healing with increased angiogenesis, which is a central pathological feature in the non-healing diabetic foot ulcer.

Supported by: MMI

## PS 102 Mechanisms and treatment of diabetic neuropathy

1173

### Cerebrovascular reactivity in patients with long-term type 1 diabetes mellitus with and without cardiovascular autonomic neuropathy

B.N. Mankovsky<sup>1</sup>, Y. Saenko<sup>2</sup>, A. Kovalenko<sup>2</sup>, O. Likhoshapko<sup>1</sup>;  
<sup>1</sup>National Medical Academy of Postgraduate Education, <sup>2</sup>Institute of Endocrinology and Metabolism, Kiev, Ukraine.

**Background and aims:** Diabetes mellitus is associated with the significantly increased risk of stroke, cognitive impairments and dementia. One of the possible underlying mechanisms of these disorders could be the impairment of cerebrovascular reactivity (CVR). Cardiovascular autonomic neuropathy (CAN) is established risk factor for stroke in patients with diabetes. However, the possible association of impairments of CVR and CAN was not studied. Therefore, the aim of this study was to investigate CVR in patients with long-term type 1 diabetes mellitus with and without CAN.

**Materials and methods:** We examined 35 subjects - 7 with type 1 diabetes mellitus and CAN (aged  $48.0 \pm 4.1$  year, diabetes duration -  $24.3 \pm 5.9$  year,  $HbA_{1c}$  -  $9.2 \pm 0.6\%$ ) (data are presented everywhere as mean  $\pm$  SEM), 13 patients with diabetes and no CAN (aged  $37.8 \pm 2.9$  year, diabetes duration -  $13.5 \pm 1.2$  year,  $HbA_{1c}$  -  $8.0 \pm 0.4\%$ ) and 15 healthy subjects as controls (aged  $47.9 \pm 2.7$  year). The subjects studied did not have any history of cerebrovascular disorders. The patients did not take any medications or products which could affect CVR. The diagnosis of CAN was based on Ewing battery of tests assessing R-R variability in electrocardiogram and diagnosis of CAN was confirmed in those patients who had more than 2 abnormal tests. The mean cerebral blood flow velocity by the middle cerebral artery (MCA) in both sides was measured by transcranial dopplerography at basal condition and immediately after the breath-holding for 30 sec and after application of the ice for 5 sec. CVR was calculated as the changes of the mean blood flow velocity by MCA immediately after the probe relative to the basal value expressed in percentage.

**Results:** Mean cerebral blood flow velocity by the right and left MCA at the basal conditions was not different in patients with type 1 diabetes with and without CAN and control group. We found that in patients with type 1 diabetes mellitus either with or without CAN there was significant decrease of CVR compared to the control group after the both probes. However, there was no significant difference in CVR after both probes between group of diabetic patients with and without CAN. CVR after the breath-holding in the right MCA was  $17.2 \pm 1.81\%$ ,  $14.8 \pm 1.49\%$  and  $28.55 \pm 3.18\%$  and in the left MCA was  $16.2 \pm 2.9\%$ ,  $14.0 \pm 1.66\%$  and  $24.8 \pm 2.87\%$  in patients with and without CAN and controls, respectively,  $p < 0.05$  for comparisons between both groups of patients with diabetes and controls. After the cold test CVR was  $17.7 \pm 2.48\%$ ,  $19.6 \pm 1.65\%$  and  $28.0 \pm 2.53\%$  at the right side and  $19.6 \pm 4.37\%$ ,  $17.6 \pm 2.69\%$  and  $24.7 \pm 1.63\%$  at the left side, in patients with diabetes with and without CAN and controls, respectively,  $p < 0.05$  for comparisons between subjects with diabetes and controls.

**Conclusion:** We may conclude that CVR is impaired in patients with long-term type 1 diabetes mellitus and these disturbances could contribute to the pathogenesis of cerebrovascular disorders in subjects with diabetes. However, CAN does not seem to play a role in the disturbances of CVR in these patients.

1174

### Na<sup>+</sup>/H<sup>+</sup>-exchanger-1 contributes to diabetic peripheral neuropathy through methylglyoxal-derived AGE formation and oxidative-nitrative stress

A.A. Obrosova<sup>1</sup>, S. Lupachyk<sup>1</sup>, P. Watcho<sup>1</sup>, H. Shevalye<sup>1</sup>, V.M. Monnier<sup>2</sup>, I.G. Obrosova<sup>1</sup>;

<sup>1</sup>Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, <sup>2</sup>Pathology, Case Western Reserve University, Cleveland, USA.

**Background and aims:** Glycolytic enzyme activities strongly depend on intracellular pH which is largely controlled by ubiquitously distributed isoform of Na<sup>+</sup>/H<sup>+</sup>-exchanger, NHE-1. Diabetes and high glucose increase NHE-1 protein content and activity. We hypothesized that NHE-1 plays an important role in diabetic peripheral neuropathy (DPN), and that NHE-1-driven upregulation of glycolysis under conditions of inhibition (endothe-



lial cells) or insufficient activation (Schwann cells) of glyceraldehyde 3-phosphate dehydrogenase leads to the diversion of the excessive glycolytic flux to methylglyoxal and  $\alpha$ -glycerophosphate, formation of methylglyoxal-derived AGE and oxidative-nitrative stress.

**Materials and methods:** In experiment 1, the severities of DPN were compared in STZ-diabetic NHE-1+/- mice (12-wk duration of STZ-diabetes) and the corresponding diabetic wild-types (C57Bl/6J background). In experiment 2, levels of 4-hydroxynonenal adducts, nitrated proteins, methylglyoxal-derived AGE (all by Western blot analyses), as well as pentosidine, carboxymethyl lysine (CML), and furosine (all by GC/MS or LC-MS/MS) were compared in the sciatic nerves of STZ-diabetic rats maintained with or without treatment with the specific NHE-1 inhibitor cariporide (10 mg kg<sup>-1</sup>d<sup>-1</sup>, 4-wk treatment after 12 wks of untreated diabetes). In experiment 3, control and STZ-diabetic rats were treated with lisinopril (1 mgkg<sup>-1</sup>d<sup>-1</sup>) or canrenone (8 mgkg<sup>-1</sup>d<sup>-1</sup>), to evaluate the role for angiotensin 2 and aldosterone in diabetes-induced NHE-1 expression.

**Results:** In contrast to the wild-types, STZ-diabetic NHE-1+/- mice did not develop sensory nerve conduction velocity deficit. They also displayed less severe motor nerve conduction velocity deficit, thermal hypalgesia, tactile allodynia, and intraepidermal nerve fiber loss. Cariporide treatment counteracted diabetes-induced accumulation of 4-hydroxynonenal adducts, nitrated proteins, and methylglyoxal-derived AGE, without affecting pentosidine, CML, or furosine levels in the sciatic nerve. Both lisinopril and canrenone reduced, but did not completely prevent, diabetes-associated increase in NHE-1 immunoreactivity in dorsal root ganglia. The measurements in peripheral nerve and spinal cord are in progress.

**Conclusion:** Angiotensin 2 and aldosterone-mediated NHE-1 upregulation contributes to nerve conduction deficit and small sensory nerve fiber dysfunction and degeneration. The contribution of NHE-1 to diabetic peripheral neuropathy is likely mediated through oxidative-nitrative stress and methylglyoxal-derived AGE formation.

Supported by: NIH DK077141, American Diabetes Association 7-08-RA-102

## 1175

### Deep breathing improves blunted baroreflex sensitivity in obese children with insulin resistance

M. Vandoni<sup>1</sup>, V. Calcaterra<sup>2</sup>, G. DeBarbieri<sup>3</sup>, D. Larizza<sup>2</sup>, R. Albertini<sup>2</sup>, A. Brigatti<sup>1</sup>, L. Correale<sup>1</sup>, M. Arpesella<sup>1</sup>, L. Bernardi<sup>3,4</sup>

<sup>1</sup>Department of Public Health, Pavia University, Italy, <sup>2</sup>Department of Internal Medicine-Paediatrics, Pavia University, Italy, <sup>3</sup>Department of Internal Medicine, Pavia University, Italy, <sup>4</sup>Folkhaelsan research Center, University of Helsinki, Finland.

**Background and aims:** Similarly to diabetes, patients with obesity show insulin resistance and autonomic abnormalities associated with increased morbidity and mortality. Although in diabetes autonomic abnormalities are conventionally considered as an organic irreversible disorder, it was previously shown that reduced baroreflex sensitivity (BRS) could be acutely corrected by slow deep breathing, suggesting that these abnormalities could be in part also functional. We then tested whether the autonomic abnormalities in young obese children result from an organic or still functional impairment (thus still reversible with appropriate interventions), and tested whether the extent of possible improvement was associated with the degree of obesity, insulin and insulin resistance.

**Materials and methods:** In 133 obese children (11.5 ± 2.9 years, 68 female 65 male, Body Mass Index (BMI) 26.9±0.4 kg/m<sup>2</sup>, mean±SEM) and 168 age-matched healthy control children, (11.5±2.5 years, 95 female 73 male, BMI 18.8±0.2 kg/m<sup>2</sup>, p<0.001) we measured fasting insulin, glucose and insulin resistance (HOMA-IR), and BRS during spontaneous (in supine and upright position) and supine controlled breathing at normal (15 breath/min) and slow-deep (6 breath/minute) breathing by simultaneous recording of beat-to-beat blood pressure (Portapres<sup>®</sup>) and electrocardiogram.

**Results:** As compared to controls, obese children showed higher systolic blood pressure (109.5±0.9 vs 103.0±1.5 mmHg p<0.001), insulin (13.1±1.1 vs 4.9±0.5 microIU/ml p<0.001) and HOMA-IR (2.52±0.22 vs 0.68±0.09 p<0.001). BRS was reduced in obese children during supine spontaneous breathing (16.3±0.8 vs 18.9±1.0 ms/mmHg, p=0.05) and even more during 15 breath/minute (12.8±0.6 vs 16.5±0.9 ms/mmHg, p=0.0011) and in upright position (8.1±0.3 vs 12.2±0.6 ms/mmHg, p=0.0001). However, during slow breathing the BRS increased markedly in both groups, and in obese children the values were close to those of control children (20.1±0.9 vs 22.7±0.9 ms/mmHg, p=0.053, NS). The extent of the improvement was proportional to the

level of insulin (r=0.24, p=0.0019) and HOMA-IR (r=0.21, p=0.0033), and only to a lower extent with BMI (r=0.13, p=0.034).

**Conclusion:** Despite evident insulin resistance and blunted resting BRS, obese children improve their BRS abnormality in response to slow breathing to an extent proportional to the metabolic abnormality. This suggests that, rather than consequence of neural anatomic lesion, the autonomic involvement is of functional origin, and likely linked to the glucose metabolism abnormalities (hyperinsulinemia and insulin resistance).

## 1176

### Repeated exposure to hypoxia improves respiratory reflexes in type 1 diabetes mellitus

L. Bernardi<sup>1,2</sup>, T. Duennwald<sup>3</sup>, D. Gordin<sup>2</sup>, A. Sandelin<sup>2</sup>, H. Gatterer<sup>3</sup>, A. Tiitu<sup>2</sup>, C. Fogarty<sup>2</sup>, M. Rosengård-Bärlund<sup>2</sup>, C. Forsblom<sup>2</sup>, M. Burtcher<sup>3</sup>, P.-H. Groop<sup>2</sup>, FinnDiane Study Group;

<sup>1</sup>Department of Internal Medicine, Pavia University, Italy, <sup>2</sup>Division of Nephrology, Department of Medicine, Helsinki University Central Hospital, University of Helsinki, Finland, <sup>3</sup>Department of Sports Science, Innsbruck University, Austria.

**Background and aims:** Intermittent hypoxic training (IHT), a technique involving exposure to 5-6 short periods (5-10min) intermittent hypoxia each day over 1-3 weeks, has successfully been used to improve adaptation to hypoxia in healthy subjects and patients with chronic bronchitis by stimulating ventilatory reflexes. In diabetes, respiratory reflexes and adaptation to hypoxia are known to be reduced. We therefore tested whether repeated hypoxic exposures over 1 day could initiate a long-lasting response that could potentially lead to better adaptation to hypoxia.

**Materials and methods:** In 15 patients with type 1 diabetes (age 36.5±1.2 yrs, [mean± SEM], diabetes duration 8.1±0.3 yrs, HbA1c 8.0±0.3 %, 13 males 2 females) we measured hypoxic and hypercapnic ventilatory responses as well as ventilatory recruitment threshold, (VRT-CO<sub>2</sub>), after 0, 3 and 6 hours of a 1-hour single bout of IHT (6-minute breathing of a 13% oxygen mixture 5 times, each time separated by a 6-minute recovery phase). The measurements were repeated on a placebo day (at least 1 week apart) in which subjects were only breathing room air (single blind protocol). The sequence of the placebo and intervention day was randomised.

**Results:** Immediately after IHT, the hypoxic and hypercapnic ventilatory responses increased from 0.23±0.04 to 0.36±0.07 L/min/%SaO<sub>2</sub> (paired t-test p<0.05), and from 0.22±0.06 to 0.39±0.107 L/min/mmHg-CO<sub>2</sub> (p<0.05), respectively, whereas the VRT-CO<sub>2</sub> dropped from 44.1±1.2 to 42.0±0.8 mmHg (p<0.05). The same directional changes remained significant after 3 hours for the hypoxic, and after 6 hours for the hypercapnic ventilatory responses. No such changes were observed during the placebo day.

**Conclusion:** In patients with type 1 diabetes repeated exposures to hypoxia during one hour induced an initial adaptation to hypoxia with improvement in the respiratory reflexes. It may be that patients with type 1 diabetes could benefit from a full (>2 weeks) IHT training.

## 1177

### Comparison of efficacy and safety of once-daily dosing and on-demand use of udenafil for type 2 diabetes patients with erectile dysfunction

K.-S. Park<sup>1</sup>, S. Park<sup>1</sup>, J. Lee<sup>1</sup>, B. Cha<sup>2</sup>, S. Park<sup>3</sup>, K. Min<sup>4</sup>, Y. Sung<sup>5</sup>, T. Kim<sup>6</sup>, I. Park<sup>7</sup>, H. Moon<sup>1</sup>

<sup>1</sup>Endocrinology & Metabolism, Department of Internal Medicine, Eulji University Hospital, Daejeon, <sup>2</sup>Endocrinology & Metabolism, Department of Internal Medicine, College of Medicine the Catholic University of Korea, Seoul, <sup>3</sup>Endocrinology & Metabolism, Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, <sup>4</sup>Endocrinology & Metabolism, Department of Internal Medicine, Eulji General Hospital, Seoul, <sup>5</sup>Endocrinology & Metabolism, Department of Internal Medicine, Ewha Woman's University School of Medicine, Seoul, <sup>6</sup>Endocrinology & Metabolism, Department of Internal Medicine, College of Medicine, Hanyang University, Seoul, <sup>7</sup>Endocrinology & Metabolism, Department of Internal Medicine, Gachon University of Medicine and Science Graduate School of Medicine, Incheon, Republic of Korea.

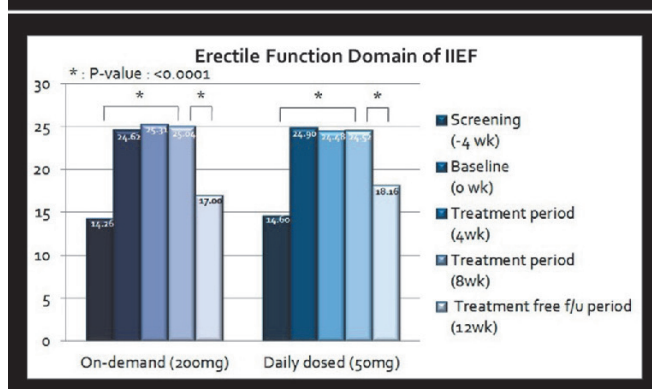
**Background and aims:** We compared the efficacy and safety between once-daily dosing and on-demand use of udenafil for type 2 diabetes patients with erectile dysfunction.

**Materials and methods:** A multi-center, randomized, open-label, parallel group, 12-week study was conducted. Patients who have shown improvement with on-demand 200mg udenafil according to Sexual Encounter Profile (SEP) diary Q2, Q3 were randomized into 200mg on-demand (n=80) or 50mg once-daily (n=81) for 8 weeks and 4 weeks of treatment-free period was followed. Udenafil was controlled in order to match the same total dose in both groups. The primary efficacy endpoint was the change of the International Index of Erectile Function (IIEF) erectile function domain (EFD) score. Secondary efficacy endpoints included changes of the SEP diary Q2, Q3, IIEF Q3, Q4, other domains of IIEF, the Global Assessment Questionnaire (GAQ), shift to normal rate (EFD $\geq$ 26). Vascular endothelial markers were assessed at baseline and after 8 weeks of treatment.

**Results:** Both groups showed significant improvement in IIEF-EFD score after 8 weeks of treatment compared to the score at screening visit ( $p<0.0001$ ). There was no significant difference between two groups. And the effect was not maintained during the treatment-free follow-up period. Similar results were observed in secondary efficacy end points. There was also no significant difference in vascular endothelial markers. Daily udenafil was well tolerated and showed non-significant tendency for fewer adverse drug reactions and adverse events.

**Conclusion:** Both methods of medication improved erectile dysfunction in diabetes patients without significant difference. Daily udenafil was not only well tolerated but also not inferior to on-demand udenafil.

## Primary Efficacy Parameter



Clinical Trial Registration Number: DA8159-DDM-IV  
Supported by: Dong-A Pharmaceutical

## 1178

**Effects of diabetes and peripheral neuropathy on joint torque development and muscle activation characteristics during stair descent**  
J.C. Handsaker<sup>1</sup>, A.J.M. Boulton<sup>2</sup>, S.J. Brown<sup>1</sup>, C.N. Maganaris<sup>1</sup>, G. Cooper<sup>3</sup>, F.L. Bowling<sup>2</sup>, N.D. Reeves<sup>1</sup>;

<sup>1</sup>IRM, Manchester Metropolitan University, <sup>2</sup>University of Manchester, <sup>3</sup>Manchester Metropolitan University, UK.

**Background and aims:** Diabetic Peripheral Neuropathy (DPN) has been shown to result in altered gait characteristics on level ground, ensuing from a decreased joint range of motion and muscular strength. An adequate rate of joint torque development and co-ordinated muscular recruitment patterns are key to a safe and stable gait cycle, particularly during the potentially dangerous task of stair descent. However, due to the lack of plantar sensitivity, it is hypothesised that DPN patients will not be able to detect key gait events to trigger these mechanisms sufficiently. This study examines the effects of diabetes and DPN on joint torque development and muscle activation characteristics during stair descent.

**Materials and methods:** 21 participants: 7 with DPN, 7 with diabetes but no/mild neuropathy (D), and 7 matched-controls descended an instrumented staircase. Three-dimensional movement, force and electromyographic (EMG) data were obtained. Values for knee and ankle joint torque generation were calculated between step contact and the peak joint moment. EMG activity was measured from a representative knee extensor (vastus lateralis), and plantar flexor (gastrocnemius) muscle, and processed in MatLab using a bespoke program to identify muscle on/off timings. Group differences were tested statistically using an analysis of variance and post-hoc tests.

**Results:** With reference to foot-step contact (FSC), the gastrocnemius muscle was activated much earlier by the D and particularly DPN groups (DPN: -202, D: -114, and Control: -61 ms;  $P=0.02$ ). In contrast, the vastus lateralis muscle was activated later and closer to the FSC by the D and DPN groups than the controls (DPN: -29, D: -39 and control: -62 ms;  $P>0.05$ ). Similar values for the rate of joint torque development were observed between groups, at the ankle (DPN: 5.2, D: 6.7 and Control: 5.8 Nm.kg.s<sup>-1</sup>;  $P>0.05$ ). In contrast, the rate of torque development at the knee showed a borderline significant difference between the groups (DPN: 6.34, D: 5.8 and Control: 7.3 Nm.kg.s<sup>-1</sup>;  $P=0.08$ ), with a slower rate of joint torque development seen in the two diabetic groups. An earlier pre-activation of the plantar flexor muscles responsible for controlling dorsiflexion of the foot upon FSC by the diabetic groups enabled generation of a similar rate of torque development to that of controls. In contrast, the delayed pre-activation of the knee extensor muscles responsible for controlling knee flexion upon FSC in the diabetic groups lead to a trend for a slower rate of torque development at the knee compared to controls.

**Conclusion:** People with diabetes, and to a greater degree those with DPN, displayed a strategy to compensate for the lack of plantar sensitivity by activating the plantarflexor muscles earlier in anticipation of FSC. In contrast, activation of the knee extensors in diabetics and DPN patients before FSC was delayed relative to controls and may reflect prioritization of the ankle plantar flexors for 'absorbing' the initial energy associated with stepping down. These differences in muscle activation and rate of joint torque development have implications for dynamic balance and the risk of falls in people with diabetes.  
Supported by: EFSD Clinical Research Grant and DRWF

## 1179

**Gabapentin/vitamins B1/B12: an efficacious and safe combination for painful diabetic peripheral neuropathy control**

M. Alpizar<sup>1</sup>, A.J. Mimenza<sup>2</sup>, R.A. Olivares<sup>3</sup>, G. Villalpando<sup>4</sup>, S.G. Aguilar<sup>2</sup>, A. Aguilar<sup>5</sup>, A.G. Garcia<sup>5</sup>, A. Torres<sup>5</sup>;

<sup>1</sup>CEDOPEC, <sup>2</sup>INNSZ, <sup>3</sup>ARKE, <sup>4</sup>CRI, <sup>5</sup>Merck, Mexico City, Mexico.

**Background and aims:** B vitamins roll in the treatment of diabetic neuropathy (DNP) is due in their effect over multiple pathways secondary to hyperglycaemia, like oxidative stress (protein kinase-C, diacylglycerol, and hexosamine pathways); likewise, to their action over nitric oxide, that explains their antihyperalgesic, antialodinic, and antinociceptive effects. The combination of thiamine and cyanocobalamin with gabapentin provide a synergistic effect, that allows a better tolerability to gabapentin, and a better analgesic effect. The main objective of this trial was to compare the efficacy for pain relief, and the safety profile of two oral medications gabapentin/vitamins B1/B12 fixed combination (GVB) vs. pregabalin (pregaba), in mild to moderate DPN, during a three months period.

**Materials and methods:** The multicentre clinical trial designed was open, randomized, and comparative, with parallel groups. Patients were followed during 12 weeks. Efficacy evaluations were performed by a numeric pain intensity scale, visual analogue scale (VAS) and Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) scale. Safety assessment was carried out through adverse events (AE).

**Results:** 323 subjects who signed an informed consent were enrolled into the study. 291 patients integrated the Intent-To-Treat (ITT) population and 239 patients the Per-Protocol (PP) population. 52% of patients received GVB, and 48% received pregaba. Numeric pain intensity scale was measured in every visit. A multivariate repeated measurements analysis was performed and every mean value during follow-up differed from baseline values, for both treatments ( $p < 0.001$ ), with no difference between treatments ( $p = 0.562$ ). Pain decrease profile was similar for both treatments. About LANSS scale, again every mean value was different from baseline measurement for both treatments ( $p < 0.001$ ), with no difference between groups ( $p = 0.730$ ). Dose scheme was titrated-up until maximum dose was reached, or to the highest dose that was tolerated. For GVB, pain relief started with the minimum dose (900 mg/day), with practically no change compared up to maximum dose (3,600 mg/day). For pregaba, there were no statistical differences among doses, which were similar from 150 to 600 mg/day. Safety was evaluated through AE evaluation. There were 291 patients who reported at least one adverse event during study. For GVB group, 65/146 patients (44.52%) presented at least one adverse event. For pregaba group, figures were 76/145 and 52.41%, respectively.

**Conclusion:** Due to the difficulty of pain handling, clinical research on neuropathic pain control is wide, including multiple therapeutic alternatives. Clinical results on pain intensity scale demonstrate that GVB is as good as

pregaba, which constitutes the standard first choice for the management of neuropathic pain in multiple guidelines. Different measurement scales were used to evaluate pain evolution, and data point out that neuropathic pain significantly decreased from a baseline score of 6.5 mm to 2.5 mm ( $p < 0.001$ ) at the end of treatment period in both treatments ( $p = 0.562$ ). Our clinical study demonstrate that GVB and pregaba have a similar safety and efficacy profile; although there was no statistically significant difference, a greater trend towards adverse events in pregaba group was observed.

Clinical Trial Registration Number: NCT01364298

Supported by: Merck

## 1180

### Nondipping is a novel associated disorder of painful diabetic polyneuropathy

V. Spallone<sup>1</sup>, R. Morganti<sup>1</sup>, C. D'Amato<sup>1</sup>, C. Greco<sup>1</sup>, F. Di Gennaro<sup>1</sup>, L. Cacciotti<sup>1</sup>, G.A. Marfia<sup>2</sup>;

<sup>1</sup>Endocrinology, Department of Internal Medicine, <sup>2</sup>Neurology, Department of Neurological Sciences, Tor Vergata University, Rome, Italy.

**Background and aims:** Sleep problems are common in painful diabetic polyneuropathy (PDPN) and the loss of the nocturnal fall in blood pressure (BP) is a recognized feature of diabetic autonomic neuropathy. We evaluated the relationship of BP circadian pattern with neuropathic pain and pain-related sleep disturbance in PDPN.

**Materials and methods:** In 105 patients with diabetes (age  $58.3 \pm 11.1$  years, diabetes duration  $15.8 \pm 12.2$  years, BMI  $29.4 \pm 4.6$  Kg/m<sup>2</sup>, 71 male, 22 with type 1 diabetes), among which 35 with PDPN (PDPN), 31 with non painful diabetic polyneuropathy (DPN<sup>+</sup>) and 39 without DPN (DPN<sup>-</sup>), neuropathic pain, sleep characteristics, risk for obstructive sleep apnoea (OSA), cardiovascular autonomic function, and BP circadian pattern were assessed using Douleur Neuropathique en 4 Questions (DN4), Brief Pain Inventory (BPI), MOS Sleep Scale (MOS-SS), Berlin Questionnaire (BQ), 4 cardiovascular autonomic reflex tests (CARTs), and 24-hour ambulatory BP monitoring (ABPM).

**Results:** PDPN group showed worse scores of MOS-SS (particularly in the items of 'sleep adequacy' and 'awaken short of breath or with headache'), and higher MOS-SS 9-item Sleep Problem Index ( $40.0 \pm 22.3$ ) compared to both DPN<sup>-</sup> group ( $22.3 \pm 17.7$ ,  $P = 0.0004$ ) and DPN<sup>+</sup> group ( $26.4 \pm 21.7$ ,  $P = 0.0155$ ). Moreover, PDPN group displayed higher night systolic BP ( $128.6 \pm 16.3$  Vs  $119.9 \pm 11.3$  mmHg,  $P = 0.009$ ) and lower day-night difference ( $\Delta$ ) in systolic BP than DPN<sup>-</sup> group ( $5.9 \pm 7.2$  Vs  $9.2 \pm 6.1$ ,  $P = 0.039$ ). No significant differences were found, instead, between DPN<sup>+</sup> group and DPN<sup>-</sup> group for MOS-SS and ABPM results, nor between PDPN group and DPN<sup>+</sup> group for CART results. The condition of non-dipping ( $\Delta$  systolic BP  $\leq 0\%$ ) was associated with PDPN ( $\text{Chi}^2 = 6.7$ ,  $P = 0.01$ ), higher CART score ( $2.9 \pm 2.9$  Vs  $1.3 \pm 1.9$ ,  $P = 0.019$ ), higher DN4-Interview score ( $4.0 \pm 2.7$  Vs  $1.7 \pm 1.7$ ,  $P = 0.0004$ ), and in PDPN patients with higher BPI pain now intensity score ( $7.2 \pm 3.6$  Vs  $2.3 \pm 2.8$ ,  $P = 0.0006$ ). The presence of high risk of OSA (i.e. 2 positive BQ categories) was not associated with PDPN, but with lower  $\Delta$  systolic BP ( $6.1 \pm 6.2$  Vs  $9.2 \pm 6.3$ ,  $P = 0.017$ ), higher MOS-SS 9-item Sleep Problem Index ( $38.9 \pm 21.9$  Vs  $23.7 \pm 19.7$ ,  $P = 0.0004$ ), and in PDPN patients with both higher pain severity score ( $6.6 \pm 2.2$  Vs  $3.6 \pm 2.4$ ,  $P = 0.0009$ ) and higher BPI pain intensity index ( $5.6 \pm 2.5$  Vs  $3.1 \pm 2.3$ ,  $P = 0.008$ ). In a stepwise regression analysis including as independent variables age, male sex, diabetes duration, BMI, orthostatic hypotension, DN4-Interview score, high risk of OSA, and MOS-SS Sleep Problem Index,  $\Delta$  systolic BP was independently related to orthostatic hypotension (step 1), DN4-Interview score (step 2), and high risk of OSA (step 3) ( $r = 0.46$ ,  $P = 0.0002$ ).

**Conclusion:** Non-dipping is a new associated disorder of PDPN and neuropathic pain is an independent determinant of BP circadian pattern. Although the pathogenetic meaning of this complex relationship remains incompletely clarified, PDPN should increasingly be regarded as a condition of high cardiovascular risk.

## 1181

### A biopsychosocial examination of factors contributing to sleep impairment in painful diabetic neuropathy

L. Thomas, D. Selvarajah, T. Cash, R. Gandhi, A. Sankar, J. Davies, S. Tesfaye; Diabetes Research Unit, University of Sheffield, UK.

**Background and aims:** Sleep is an important component of good general health and well being. It is also well recognised that sleep disturbance in painful diabetic neuropathy (DN) is common and leads to substantial impairments in quality of life. However, our current knowledge regarding the relationship between sleep, mood disorders and physical functioning in painful DN is limited. The aim of this study was thus to assess the contributions of the diabetes state, neuropathic pain and psychological factors to self-reported sleep disturbance in patients with painful DN.

**Materials and methods:** 113 patients with confirmed painful DN participated in a burden of illness assessment study. This study adopted a multidimensional approach to test a biopsychosocial model to understanding sleep disturbance in painful DN. Each patient underwent structured interviews, detailed clinical assessments and completed self-reported measures of pain severity (Brief Pain Inventory DN [BPI-DN]), pain acceptance (Chronic Pain Acceptance [PCA] questionnaire), illness beliefs (Pain Catastrophizing Scale [PCS]), affective distress (Hospital Anxiety and Depression [HADS] questionnaire) and sleep impairment (Medical Outcomes Study-Sleep [MOS-Sleep] Scale). In addition, patients provided information regarding their age, gender, marital status, social deprivation score, medication use, diabetes and other medical co-morbidities.

**Results:** 108 patients (96%) reported moderate to severe levels of sleep impairment which affected all subscales of the MOS-sleep questionnaire (sleep disturbance, adequacy quality and somnolence). A set of predictor variables was established using bivariate correlation analyses. Female gender ( $p = 0.005$ ), pain severity (BPI-DN  $p < 0.001$ ), affective distress (HADS-Anxiety and HADS-Depression  $p < 0.001$ ) and illness beliefs (PCS  $p < 0.001$ ) were all correlated positively with MOS-Sleep score, while age ( $p = 0.001$ ), pain acceptance (PCA  $p < 0.001$ ) and diabetic comorbidities ( $p = 0.002$ ) were correlated negatively with MOS-Sleep. SNRIs ( $p = 0.01$ ), anticonvulsants ( $p = 0.04$ ) and opiates (0.01) but not tricyclic antidepressants ( $p = 0.32$ ) use were correlated with greater sleep impairment. No significant correlation with marital status, deprivation score and duration of pain or diabetes. Hierarchical multiple regression confirmed that higher anxiety symptom scores ( $\beta = 1.02$ ), greater pain severity ( $\beta = 1.86$ ), less pain acceptance ( $\beta = -0.34$ ) and female gender ( $\beta = 6.30$ ) contributed independently to higher sleep disturbance. This final regression model taking accounted for 57.0% of the variance in MOS-Sleep scores ( $F = 8.65$ ,  $p < 0.001$ ).

**Conclusion:** A very high prevalence of sleep disturbance was found study in this patient population of patients with painful DN. Women were also found to be more susceptible to sleep disturbance. Finally, this study has identified key factors that contribute to sleep disturbance in painful DN and this has implications for the development of rational, empirically based pain management strategies to improve sleep disturbance. Studies are now required to explore the impact of interventions that target pain and anxiety symptoms on sleep outcomes in painful DN.

## 1182

### Serotonin pain facilitation through 5-HT<sub>3</sub> receptor in diabetic neuropathic pain

M.A. Silva, I. Tavares, C. Morgado; Faculty of Medicine of Porto and IBMC, Department of Experimental Biology, University of Porto, Portugal.

**Background and aims:** Diabetic neuropathic pain (DNP) is a chronic and debilitating pain condition that affects about one fifth of diabetic patients. DNP is poorly responsive to the pain killers currently used to treat chronic pain and is only moderately relieved by the use of anticonvulsants and antidepressants. Serotonin selective reuptake inhibitors (SSRIs) were shown to elicit low analgesic effects in DNP, which is difficult to explain considering the pain inhibitory actions of serotonin at the spinal cord in normal conditions. This finding suggests that during DNP the pain modulatory effects of serotonin are likely to be impaired. In acute pain conditions, serotonin inhibits pain transmission by binding to the 5-HT receptors present at spinal dorsal horn, but, it was recently shown, that in a chronic pain situation serotonin may facilitate pain through its action on spinal 5-HT<sub>3</sub> receptor. This mechanism is likely to account to DNP and may explain the low antinociceptive effect of



the SSRIs. Thereafter, this study aimed to evaluate the role of spinal 5-HT<sub>3</sub> receptor (5-HT<sub>3</sub>R) in mediating diabetic-induced mechanical hypersensitivity. **Materials and methods:** Male wistar rats were rendered diabetic by an intraperitoneal injection of streptozotocin (STZ, 60 mg/kg body weight) and the control (CTR) animals received only the vehicle. Three weeks post-injection a catheter was implanted in the lumbar subarachnoid space and animals were allowed to recover for a week. At 4 weeks post-injection mechanical nociception was behaviourally evaluated using the paw pressure test (Randall-Selitto test) in STZ and control rats receiving intrathecal infusions of saline or 5-HT<sub>3</sub> receptor antagonist (30 fmol, Y-25130 hydrochloride).

**Results:** STZ rats presented significantly increased glycaemia (STZ: 520 ± 58 mg/dl; CTR: 127 ± 17 mg/dl,  $p < 0.0001$ ; independent sample t test) and haemoglobin A1C (STZ: 12.0 ± 0.8; CTR: 4.9 ± 0.2,  $p < 0.0001$ ; independent sample t test), along with decreased paw pressure thresholds (STZ: 89 ± 6 g; CTR: 140 ± 1 g,  $p = 0.0006$ ; independent sample t test). The intrathecal delivery of 5-HT<sub>3</sub>R antagonist induced a significant increase in the paw pressure thresholds in the STZ rats whereas no effects were detected in the control rats (Untreated STZ\*: 89 ± 6 g; STZ+Y-25130: 139 ± 7 g; Untreated CTR: 140 ± 1 g; CTR+Y-25130: 139 ± 6 g; \* $p < 0.01$  vs all other groups, One-Way ANOVA followed by tukey post hoc test). The intrathecal infusion of saline did not change the pain behaviour in any experimental group.

**Conclusion:** These results demonstrated that 5-HT<sub>3</sub> receptor activation facilitate the spinal pain transmission in DNP. The increased serotonin level at the synaptic cleft induced by SSRIs administration may elicit pro-nociceptive effects through the activation of 5-HT<sub>3</sub> receptor that may be masking the inhibitory actions of the others spinal 5-HT receptors. New serotonin-based pain therapies for DNP should consider the particular pain facilitating role of 5-HT<sub>3</sub>R.

Supported by: FCT project PTDC/SAU-NSC/110954/2009

## PS 103 Diabetic foot: risk factors

### 1183

#### Toe-brachial index is a better index for establishing the severity of PAD than ankle-brachial index in patients with diabetes mellitus

X. He, Z. Huang, A. Chen, Y. Li;

The department of Endocrinology, The first affiliated hospital of Sun Yat-sen University, Guangzhou, Guangdong Province, China.

**Aims:** Ankle-brachial index (ABI) is a useful clinical test for establishing the severity of peripheral arterial disease (PAD). However, there are limitations to this method when conducted on people with diabetes mellitus because of the presence of medial artery calcification. As an alternative to the ankle brachial index, toe-brachial index (TBI) has been recommended to assess the arterial blood supply to the foot. This study evaluated the relevance of ABI and TBI in patients with diabetes, and aimed to obtain more information about when toe-brachial index should be assessed on patients with diabetes mellitus.

**Methods:** 127 subjects with diabetes underwent ABI, TBI (measured by Doppler ultrasound) and low extremity arterial duplex ultrasound (as golden standards) from August 2011 to February 2012. Kruskal-Wallis, one-way analysis of variance was used to compare demographic and clinical parameters of subjects stratified by ABI. Pearson's correlation coefficient was used to assess the linear relationship between two indexes. Receiver operating characteristic (ROC) curve analysis was used to evaluate the sensitivity and specificity of TBI in patients with ABI ≥ 1.3.

**Results:** Of 127 total patients with diabetes, 62.2% had PAD. All patients were categorized into three groups: reduced (ABI ≤ 0.9), normal (0.9 < ABI < 1.3), and elevated (ABI ≥ 1.3). There were no significant differences in demographic and clinical parameters across those three groups. By linear correlation, TBI showed a significant positive correlation with ABI in subjects with an ABI < 1.3. In contrast ( $r = 0.449$ ,  $P = 0.003$ ). In contrast, TBI was not correlated with ABI in patients with an ABI ≥ 1.3. When patients are categorized according to ABI and TBI, there is also good agreement between the tests when ABI is reduced or normal (64.3%), but not when ABI is elevated (24.7%). In patients with ABI ≥ 1.3, ROC analysis showed that sensitivity and specificity of TBI < 0.6 were 76.2% (95%CI, 69.2 to 84.1%) and 88.4% (95%CI, 83.7 to 94.2%).

**Conclusion:** For those patients with high ABI (≥ 1.3), which means overt medial artery calcification, TBI is a better clinical test for establishing the severity of PAD than ABI.

### 1184

#### The role and the potential ways by which psychological stress impedes diabetic foot ulcer healing

L. Vileikyte<sup>1</sup>, L. Campbell<sup>2</sup>, B. Jiun-Shen<sup>3</sup>, A. Boulton<sup>1</sup>, M. Hardman<sup>2</sup>;

<sup>1</sup>Medicine, University of Manchester, UK, <sup>2</sup>Life Sciences, University of Manchester, UK, <sup>3</sup>Psychology, Ohio University, Columbus, USA.

**Background and aims:** Research indicates that psychological stress (PS)-induced immunomodulation delays acute wound repair. Here we explored the potential role of PS in the more complex diabetic foot ulcer (DFU)-healing paradigm.

**Materials and methods:** Ninety three type 2 DM patients (84% male; mean age 57yrs) with plantar DFU (University of Texas Classification: 69% grade 1A; 11% 1B; 16% 2A; and 4% 2B) completed baseline self-report measures of generalized (Perceived Stress Scale, PSS; Hospital Anxiety and Depression Scale, HADS; and State-Trait Anger Expression Inventory, STAXI) and DFU-specific PS (NeuroQoL-Interpersonal Burden (NeuroQoL-IP) and Patient Interpretation of Neuropathy (PIN) Scales: PIN-Amputation Worry and PIN-Anger at Docs). DFU-specific biomarkers (IL-6, IL-1β, MMP-2 and MMP-9) were determined via quantification of immunohistochemical tissue localization and/or normalized biopsy gene expression. Systemic biomarkers (IL-6 and IL-1β) were measured from patient serum via ELISA.

**Results:** Bivariate analyses revealed multiple measures of increased generalized and DFU-specific emotional distress were associated with: a) decreased local IL-1β at baseline: HADS-Depression ( $r = -0.27$ ;  $p < 0.001$ ) and NeuroQoL-IP ( $r = -0.38$ ;  $p < 0.01$ ); b) decreased MMP-9: HADS-Depression ( $r = -0.29$ ;  $p < 0.05$ ); PIN-Worry ( $r = -0.34$ ;  $p < 0.05$ ) and PIN-Anger ( $r = -0.37$ ;  $p < 0.01$ ); c) increased MMP-2: PIN-Worry ( $r = 0.32$ ;  $p < 0.05$ ) and PIN-Anger ( $r = 0.31$ ;  $p < 0.05$ ). STAXI was associated with higher levels of baseline systemic IL-6 ( $r = 0.32$ ;  $p < 0.01$ ). Intriguingly, greater than 80% DFU area reduction at 6

weeks was less likely in patients reporting more severe PIN-Worry ( $r=-0.36$ ;  $p<0.01$ ) and PIN-Anger ( $r=-0.30$ ;  $p<0.01$ ) and associated with higher baseline levels of systemic IL-6 ( $r=-0.29$ ;  $p<0.05$ ) and local MMP-2 ( $r=-0.32$ ;  $p<0.05$ ).

**Conclusion:** These preliminary data identify potential psychological stress-induced biomarkers linking stress to DFU chronicity.

*Clinical Trial Registration Number:* 5-R01-DK07-1066-05

*Supported by:* NIDDK

## 1185

### Leg ulcers predictors in patients with necrobiosis lipidica and diabetes mellitus type 1

D.A. Semenova<sup>1</sup>, S.A. Prokofev<sup>2</sup>, E.A. Repina<sup>2</sup>, A.U. Tokmakova<sup>1</sup>, S.M. Stepanova<sup>2</sup>, E.V. Matushevskaya<sup>3</sup>, O.N. Bondarenko<sup>1</sup>, U.U. Orlov<sup>4</sup>; <sup>1</sup>Department of Podiatric Medicine and Surgery, Endocrinological Research Centre, <sup>2</sup>Laboratory of Immunology and Genetics, Endocrinological Research Centre, <sup>3</sup>Postgraduate Education, Federal Medicobiologic Agency, <sup>4</sup>Department of Ultrasound, Endocrinological Research Centre, Moscow, Russian Federation.

**Background and aims:** Necrobiosis lipidica (NL) is a chronic inflammatory skin disease with unknown aetiology. The aim of this study was to investigate new diagnostic methods and prognostic factors of leg ulceration in patients with necrobiosis lipidica and diabetes mellitus type 1.

**Materials and methods:** 36 patients with diabetes mellitus type 1 were examined. They were divided into the following two groups: patients with diabetes mellitus type 1 and necrobiosis lipidica (group 1), patients with diabetes mellitus type 1 without necrobiosis lipidica (group 2). The expression of Toll-like receptor type 2 and Toll-like receptor type 3 on monocytes and neutrophils was determined by flow cytometry using monoclonal antibodies. Results were compared with the data of ultrasound skin morphometry and clinical characteristics of the patients.

**Results:** There were 3 males and 21 females in group 1; 2 males and 10 females in group 2. Median age, diabetes mellitus duration, HbA<sub>1c</sub> in group 1 and group 2 were respectively 26 years, 14 years, 9.4 % and 28 years, 15 years, 9.0 %. Intensities of microvascular complications (diabetic retinopathy and diabetic nephropathy) were comparable among the 2 groups. 29% of patients with necrobiosis lipidica had documented chronic ulcers in dermatosis foci. The median duration of chronic wound existence was 5 month [3; 7]. Median thickness of derma in necrobiosis lipidica foci according to ultrasound skin morphometry was 17mm [16;18], median thickness of derma in symmetrical zones of visibly normal skin was 14mm [13; 16]. Derma in necrobiosis lipidica foci was significantly thicker than in normal skin ( $p=0.03$ ). Lower echogenicity and vague border between derma and subcutaneous fat were present in necrobiosis lipidica foci, which may be caused by granulomatous inflammation, destruction of dermal fibres, necrobiosis of connective tissue. It was found that Toll-like receptor type 2 expression on monocytes and fluorescence intensity were significantly decreased in group 1 (59/59) in comparison with group 2 (79/71) ( $p=0.026$  and  $p=0.019$ ). These markers had significant negative correlation with ulceration in patients with necrobiosis lipidica ( $R=-0.84$   $p=0.05$  and  $R=-0.7$ ,  $p=0.046$ ) and thickness of derma measured by ultrasound ( $R=-0.83$ ,  $p=0.003$ ). Toll-like receptor type 3 expression on neutrophils (%) and fluorescence intensity also were significantly lower in group 1 (33/49) in comparison with group 2 (62/53) ( $p=0.04$  и  $p=0.02$ ).

**Conclusion:** Evaluation of the expression of Toll-like receptor type 2 on monocytes and Toll-like receptor type 3 on neutrophils can be used for necrobiosis lipidica risk stratification, predicting disease severity and improving management strategy in patients with diabetes mellitus type 1.

## 1186

### Percutaneous recanalisation in diabetics with critical limb ischaemia: comparison of angiographic and clinical results according to three different classification systems

E. Iacopi<sup>1</sup>, I. Bargellini<sup>2</sup>, A. Coppelli<sup>1</sup>, R. Cervelli<sup>2</sup>, L. Rizzo<sup>1</sup>, R. Cioni<sup>2</sup>, A. Piaggini<sup>1</sup>;

<sup>1</sup>Diabetic Foot Section - Department of Medicine, University of Pisa,

<sup>2</sup>Interventional Radiology Section - Radiology Department, University of Pisa, Italy.

**Background and aims:** To evaluate the ability of three different angiographic classifications of describing the actual pattern of diabetic macro-angiopathy

(DMA) and to test their prognostic value towards clinical outcomes of diabetic foot (DF) patients with critical limb ischaemia (CLI).

**Materials and methods:** We traced back all DF patients with CLI submitted to percutaneous transluminal angioplasty (PTA) in the Section of Interventional Radiology of our Hospital in the years 2009 - 2010. DF lesions were classified according to the Texas University Score System (TUSS). DMA severity was assessed according to three different classification systems based on angiographic pattern: the *Trans Atlantic Society Consensus II* (TASC II), the *Joint Vascular Society Council* (JVSC), and the morphological classification. We eventually correlated the clinical results (healing rate, healing time, rate and level of amputations), with the severity of vascular involvement before and after the revascularization procedures as expressed by the three different classifications.

**Results:** We evaluated 202 consecutive PTA performed in 166 diabetic patients (Male/female 115/51, mean age 72.8±9.8 yrs, duration of diabetes 20.5±12.1 yrs, HbA<sub>1c</sub> 7.8±1.8%). 43.6% of FL were scored as D3 according to TUSS and involved the forefoot in 78.7% of the cases. TASC II was not applicable in 55.4% of patients before and in no patient after PTA because of the absence of supra-popliteal arterial stenosis. The mean score assigned with JVSC was 7.8±1.7 before and 4.8±2.3 ( $p<0.01$ ) after PTA while according to the Graziani classification we observed a pre-procedural mean score of 4.8±0.9 and a post-procedural one of 1.4±0.5 ( $p<0.01$ ). The immediate technical success rate of PTAs was 93.6%. Patients were followed up for 13.4±9.7 months. The complete healing of lesions was reached by 66.8% of patients with a healing time of 28.4±23.7 weeks, while 3.9% of them needed to undergo a major amputation. Patients healed with a minor amputations were 47%. Among all the clinical and angiographic variables included in the analysis, only JVSC scores were significantly associated to the clinical outcome ( $p=0.04$ ).

**Conclusion:** TASC II is inadequate to describe vascular involvement in the of diabetic patients with CLI. While both the other classifications are effective in describing both basal and post-PTA conditions of DMA, only JVSC is a reliable predictor of outcome in DF patients with CLI.

## 1187

### Risk factors for cardiovascular mortality in patients with diabetic foot ulcers: a longitudinal study

T. Tay<sup>1</sup>, L. Cho<sup>1</sup>, J. Khoo<sup>1</sup>, R. Chen<sup>1</sup>, S. Barbier<sup>2</sup>, J. Ng<sup>1,2</sup>;

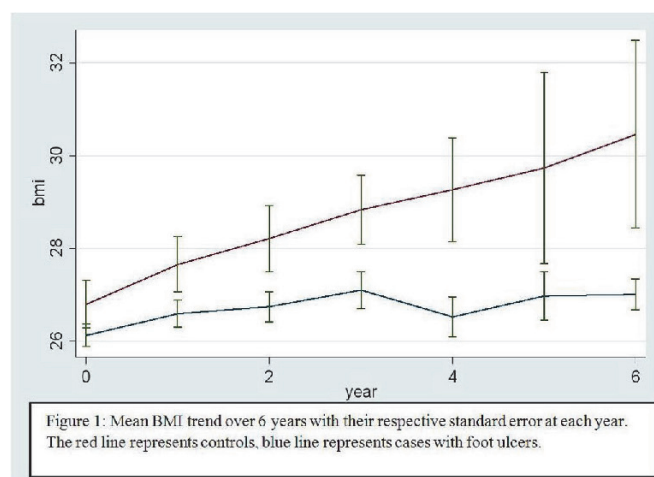
<sup>1</sup>Changi General Hospital, Singapore, <sup>2</sup>Duke-NUS, Singapore.

**Background and aims:** Diabetic foot ulcers has a prevalence of 10% per year and is an important cause of morbidity and mortality for patients with diabetes mellitus. The cardiovascular mortality are as high as 44% after 5 years in these patients. There are few studies looking at metabolic parameters which could lead to the increased mortality and cardiovascular morbidity after the onset of diabetic foot ulcers. We aim to evaluate the changes in metabolic parameters that occur after the development of diabetic foot ulcers which may explain why these subjects have a higher risk of cardiovascular mortality.

**Materials and methods:** A longitudinal study of 193 new onset diabetic foot ulcer subjects and 386 type 2 diabetes mellitus matched controls with no foot ulcers attending similar diabetes services at a tertiary regional hospital in Singapore were studied and followed annually for 6 years from January 2004 to March 2010. Their HbA<sub>1c</sub>, lipid profile, urine microalbumin, blood pressure and body mass index (BMI) were monitored yearly.

**Results:** The baseline characteristics of the cases and controls were similar. Glycated hemoglobin (A1c) were similar in cases and controls at 8.2±2% at baseline and 8.0±1.6% at the end of follow-up ( $p=0.53$ ). In contrast, BMI increased steadily and significantly in subjects with new onset foot ulcers over the course of follow-up (Figure 1). The mean percentage increase in BMI after 6 years was 5.5%, compared with controls of 1.4% ( $p=0.01$ ). The mortality rate was significantly higher in subjects with foot ulcers compared with controls after 6 years, despite no difference in trends in A1c, lipid profile and blood pressure (7.3% vs 3.1% respectively,  $p=0.02$ ).

**Conclusion:** Patients are at risk of being overweight and obese after development of diabetic foot ulcers. Early mobilization of patients with diabetic foot ulcers to encourage independence would be an important goal. Controlling BMI, besides optimization of DM, hypertension and hyperlipidemia, is important for reducing cardiovascular morbidity and mortality. Weight neutral treatment such as GLP-1 agonists might play a role in maintaining glycemic control as well as BMI. More follow-up studies would be needed to confirm the outcome of diabetic adults who gain weight after developing foot ulcers.



## 1188

### Vascular, sensory and renal function in patients attending community-based foot screening: results from the West of Ireland Diabetes Foot Study

L. Hurley<sup>1</sup>, A.P. Garrow<sup>2</sup>, L. Kelly<sup>1</sup>, L.G. Glynn<sup>3</sup>, S.F. Dinneen<sup>1,4</sup>;

<sup>1</sup>Diabetes Centre, University Hospital Galway, Ireland, <sup>2</sup>School of

Social Work, Psychology and Public Health, University of Salford, UK,

<sup>3</sup>Department of General Practice, National University of Ireland, Galway,

Ireland, <sup>4</sup>Department of Medicine, National University of Ireland, Galway, Ireland.

**Background and aims:** Annual diabetic foot screening is recommended with subsequent care determined by the patients' assigned risk status. With an increasing emphasis on primary care based diabetes care, general practice is the ideal setting for this screening. This study aimed to describe the prevalence of risk factors for diabetic foot ulceration in Irish General Practice.

**Materials and methods:** All patients with diabetes attending 12 general practices were invited for screening. Examination comprised 10g monofilament cutaneous pressure perception (CPP), vibration perception threshold (VPT) and a composite modified neuropathy disability score (mNDS). Vascular status was assessed by palpation of pedal pulses (PP) and doppler waveform assessment. The most recent creatinine result was used to calculate eGFR [using the abbreviated MDRD equation:  $186 \times (\text{Creat} / 88.4)^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$ ] and hence chronic kidney disease (CKD) stages.

**Results:** Of the total invited for screening 563 (68%) patients attended. Mean age was 64 years (SD13), 60% were male, 90% had type 2 diabetes and mean duration of diabetes was 7.7 years. Mean HbA<sub>1c</sub> was 7.3% (SD1.4), serum creatinine 89.6μmol/l (SD31.5) and eGFR 78.4ml/min/1.73m<sup>2</sup> (SD24.4). A review of medical records revealed 51% had hypertension, 26% cardiovascular disease, 3.7% reported a prior foot ulcer and 1.6% an amputation. On examination 25% had impaired CPP, 23% impaired VPT and 24% abnormal mNDS. All three modalities were abnormal in 10% of patients. 18% had ≥2 PP absent and 39.6% had abnormal doppler waveform assessment defined as a mono- or biphasic pulse. Those with abnormal CPP, VPT or mNDS had a significantly lower mean eGFR than those with normal sensory function: 74.1 vs 79.9ml/min/1.73m<sup>2</sup> for CPP ( $p=0.02$ ), 68.4 vs 80.2ml/min/1.73m<sup>2</sup> for VPT ( $p<0.001$ ) and 72.2 vs 80.5ml/min/1.73m<sup>2</sup> for mNDS ( $p<0.001$ ). Renal function was also poorer in patients with abnormal PP palpation or doppler waveform compared to those with normal vascular function: eGFR 70.2 vs 80.2ml/min/1.73m<sup>2</sup> ( $p<0.001$ ) and 71.6 vs 82.9ml/min/1.73m<sup>2</sup> ( $p<0.001$ ) respectively. Stage 3 and 4 CKD was prevalent in 19.3% and 2% of patients. There was a stepwise increase in the prevalence of all sensory and vascular risk factors with advancing CKD (Table 1).

**Conclusion:** Our data show the extent of sensory dysfunction and vascular impairment in a representative sample of diabetic patients in general practice and supports existing data showing a relationship between these risk factors and CKD. The use of eGFR may facilitate the identification of at-risk patients earlier in the disease process.

### Prevalence of sensory and vascular risk factors in CDK groups

Table 1	Normal: Stage 1 CKD	Mild: Stage 2 CKD	Moderate/Severe: Stage 3 or 4 CKD	p-value
n(%)				
Abnormal CPP	31/157 (19.7%)	66/238 (27.7%)	35/107 (32.7%)	0.05
mNDS>6	26/157 (16.6%)	59/238 (24.8%)	40/107 (37.4%)	0.001
VPT>25	13/79 (16.5%)	34/151 (22.5%)	25/64 (39.1%)	0.005
Abnormal PP	17/157 (10.8%)	42/238 (17.6%)	31/107 (29.0%)	0.001
Abnormal doppler waveform	45/157 (28.7%)	91/238 (38.2%)	65/107 (60.7%)	<0.001

Supported by: DFI HRB MRCG

## 1189

### Preserved microcirculation in the foot after intensified insulin treatment in patients with type 1 diabetes mellitus

B. Rathsmann<sup>1,2</sup>, T. Nyström<sup>1,3</sup>, K. Jensen-Ustad<sup>1,4</sup>;

<sup>1</sup>Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, <sup>2</sup>Sach's Children's Hospital, Södersjukhuset, <sup>3</sup>Section of Internal Medicine, Södersjukhuset, <sup>4</sup>Section of Clinical Physiology, Södersjukhuset, Stockholm, Sweden.

**Aims:** We sought to investigate microvascular function, by using laser doppler flowmetry and iontophoresis, in the foot of patients with type 1 diabetes after intensified conventional insulin treatment (ICT) compared to patients with standard insulin treatment (ST).

**Methods:** Patients previously participating in the Stockholm Diabetes Intervention Study (SDIS) were reinvestigated after  $12.6 \pm 0.5$  years, with a single point iontophoresis, using a laser doppler technique after stimulation with topically applied acetylcholine (2%), nitroprusside (2%), and capsaicin (2%). The microvascular blood flow, for each of the vasoactive substances used, was calculated as a quotient between the maximal blood flow after stimulation and the basal blood flow. The ankle brachial index (ABI) was also measured. In the SDIS, patients were randomized to ICT or ST, for 7.5 years. In the present study, patients were no longer randomized and treatment was decided by routine care.

**Results:** 35 patients (ICT group, 79.5%) and 37 (ST group, 71.2%) were included from the original SDIS study. The mean age was for the ICT group  $30 \pm 8$  and for the ST group  $32 \pm 8$  years ( $P=0.29$ ). 7.5 years after randomization mean HbA<sub>1c</sub> was reduced from  $9.5 \pm 1.3\%$  to  $7.1 \pm 0.6\%$  in the ICT group, and from  $9.4 \pm 1.4\%$  to  $8.3 \pm 0.7\%$  in the ST group,  $P<0.001$ . 12.6 years after randomization mean HbA<sub>1c</sub> was still significantly lower in the ICT group  $7.4 \pm 0.8\%$  vs. the ST group  $8.3 \pm 0.9\%$ ,  $P<0.001$ . ABI did not differ between the groups,  $1.1 \pm 0.1$  (ICT) vs  $1.1 \pm 0.1$  (ST),  $P=0.7$ . Basal microvascular blood flow did not differ between groups, however, stimulated blood flow was attenuated in the ST vs. ICT group regarding; acetylcholine ( $5.3$  vs  $8.1$ ;  $P=0.004$ ), nitroprusside ( $5.6$  vs  $8.1$ ;  $P=0.031$ ), and capsaicin ( $3.4$  vs  $5.0$ ;  $P=0.005$ ). A significant correlation between mean HbA<sub>1c</sub> during the 7.5 years randomized study was demonstrated between acetylcholine ( $r=0.42$ ,  $P<0.05$ ), nitroprusside ( $r=0.26$ ,  $P<0.05$ ) and capsaicin ( $r=0.28$ ,  $P<0.05$ ). However, in multivariate regression analysis adjusted for duration of diabetes, age, blood pressure, BMI, smoking and HbA<sub>1c</sub> (base-line); mean HbA<sub>1c</sub> during the 7.5 years' randomized study was independently associated with an increase in microvascular blood flow after acetylcholine ( $P=0.001$ ), but not for nitroprusside ( $P=0.115$ ), nor for capsaicin ( $P=0.055$ ).

**Conclusion:** Improvement of hyperglycaemia in patients with type 1 diabetes preserves peripheral microcirculation in a long time perspective. This preservation, of endothelial-dependent microvascular blood flow, seems to be due to lowering hyperglycaemia, independent of other classical risk-factors for endothelial-dysfunction.



## 1190

**Effects of biofeedback on plantar pressure distribution in diabetic patients with peripheral neuropathy**

Z. Pataky<sup>1</sup>, D. De León Rodríguez<sup>2</sup>, L. Allet<sup>3,4</sup>, J. Philippe<sup>5</sup>, J.-P. Assal<sup>6</sup>, C.-A. Hauert<sup>2</sup>, A. Golay<sup>1</sup>;

<sup>1</sup>Division of Therapeutic Education for Chronic Diseases, University Hospitals of Geneva, <sup>2</sup>Faculty of Psychology and Educational Sciences, University of Geneva, <sup>3</sup>Health Care Directorate, University Hospitals of Geneva, Switzerland, <sup>4</sup>Department of Physical Therapy, University of Applied Sciences, Geneva, Switzerland, <sup>5</sup>Service of Endocrinology, Diabetes and Nutrition, University Hospitals of Geneva, Switzerland, <sup>6</sup>Foundation for Research and Training in Patient Education, Geneva, Switzerland.

**Background and aims:** Plantar pressure reduction is essential for diabetic foot ulcer healing. Our aim was to evaluate the impact of a new walking strategy learned by biofeedback on plantar pressure distribution under both feet in patients with diabetic peripheral neuropathy.

**Materials and methods:** Terminally augmented biofeedback has been used for foot off-loading training in 21 patients with diabetic peripheral neuropathy. The biofeedback technique was based on a subjective estimation of performance and objective visual feedback following walking sequences. The patient was considered to have learned a new walking strategy as soon as the peak plantar pressure (PPP) under the previously defined at-risk zone was within a range of 40–80% of baseline PPP, in 70% of the totality of steps and during 3 consecutive walking sequences. The PPP was measured by a portable in-shoe foot pressure measurement system (PEDAR<sup>®</sup>) at baseline (T0), directly after learning (T1) and at 10-day retention test (T2). We used ANOVA with Bonferroni post-hoc testing to determine differences between PPP at different phases of the learning procedure and retention tests.

**Results:** The PPP under at-risk zones decreased significantly at T1 ( $165 \pm 9$  kPa,  $p < 0.0001$ ) and T2 ( $167 \pm 11$ ,  $p = 0.001$ ), as compared to T0 ( $242 \pm 12$  kPa) without any increase of the PPP elsewhere. At the contra-lateral foot (not concerned by off-loading), the PPP was slightly higher under the lateral midfoot at T1 ( $68 \pm 8$  kPa,  $p = 0.01$ ) and T2 ( $65 \pm 8$  kPa,  $p = 0.01$ ), as compared to T0 ( $58 \pm 6$  kPa).

**Conclusion:** The foot off-loading by biofeedback lead to a safe and regular plantar pressure distribution without inducing any new “at-risk” area under both feet.

## PS 104 Genes and chronic complications

## 1191

**FoxO1 acetylation is correlated to metabolic control and expression of inflammatory factors in atherosclerotic plaques from subjects with and without diabetes**

M. Federici<sup>1</sup>, M. Cardellini<sup>1</sup>, E. Martelli<sup>2</sup>, V. Casagrande<sup>1</sup>, F. Davato<sup>1</sup>, A. Valentini<sup>1</sup>, O. Porzio<sup>1</sup>, D. Lauro<sup>1</sup>, A. Ippoliti<sup>1</sup>, R. Lauro<sup>1</sup>, R. Menghini<sup>1</sup>;

<sup>1</sup>Dept. of Internal Medicine, <sup>2</sup>University of Tor Vergata, Rome, Italy.

**Background and aims:** Recent evidences suggested that dynamic interactions between monocyte/macrophage and activated endothelium are regulated via FoxO1-dependent transcription of TLR4 and VCAM1, pinpointing FoxO1 as a pivotal element in the onset of atherosclerosis. FoxO1 function is fine tuned through post-translational modifications, particularly lysine acetylation. Transgenic models carrying constitutively deacetylated Foxo1 in mice (FoxO1KR/KR) showed improved hepatic lipid metabolism and decreased macrophage inflammation, but increased atherosclerosis on a LDLR knockout background. Given these paradoxical results, our aim was to investigate the activity of FoxO1 in human atherosclerosis.

**Materials and methods:** In human atherosclerotic carotid plaques biobank (n=100) we have determined mRNA expression of FoxO1, VCAM-1, TLR4, MMP9, TNF-alpha and CCL2 by quantitative real time PCR (qPCR) normalizing to 18S and analysing with the deltadeltaCT method. In protein extracts we have analyzed the levels of VCAM-1, FoxO1, total FoxO1 Lysine acetylation (FoxO1Ac, 7 Lysine residues acetylated) and FoxO1 acetylation mediated specifically by SirT1 on Lysine 241 (FoxO1-SirT1Ac), normalizing to tubulin and FoxO1 levels. mRNA expression levels were normalized using logarithmic transformation.

**Results:** In atherosclerotic plaques we found that FoxO1Ac was positively correlated with the levels of several inflammatory markers, such as VCAM-1 ( $R = 0.53$   $p < 0.0001$ ), TLR4 ( $R = 0.44$   $p = 0.002$ ) and MMP9 ( $R = 0.44$   $p < 0.0001$ ) mRNA expression, whereas FoxO1-SirT1Ac was mildly correlated only with VCAM-1 mRNA ( $R = 0.25$   $p = 0.005$ ) and VCAM-1 protein levels ( $R = 0.22$   $p = 0.008$ ). Moreover, FoxO1 mRNA expression resulted in correlation with VCAM-1 ( $R = 0.73$   $p < 0.001$ ), TLR4 ( $R = 0.78$   $p < 0.001$ ), TNF-alpha ( $R = 0.75$   $p = 0.002$ ), CCL2 ( $R = 0.7$   $p < 0.001$ ) mRNA expression and with the FoxO1Ac levels ( $R = 0.34$ ,  $p = 0.005$ ). Metabolic analysis indicate that FoxO1Ac is correlated with Fasting Plasma Glucose ( $R = 0.23$   $p = 0.039$ ), therefore we divided our samples in 4 groups according to the metabolic status (Normal Glucose Tolerance n=35, Impaired glucose Tolerance n=31, Type 2 Diabetes Mellitus with new diagnosis and no treatment n=18, Type 2 Diabetes Mellitus under treatment=16); by ANOVA statistical analysis, we found that the degree of metabolic status is positively correlated with FoxO1Ac ( $p = 0.002$ ) whereas the FoxO1 mRNA expression is similar in the different groups.

**Conclusion:** Our data suggest that FoxO1 is differently regulated in atherosclerotic tissues. Hyperglycemia and impaired glucose metabolism were correlated with FoxO1 acetylation status, possibly affecting FoxO1 activity. Previous experimental data suggested that FoxO1 can exert both pro- and anti-inflammatory actions in vascular cells depending on its post-translational acetylation status. In this study, we found that Acetylated FoxO1 is associated to systemic hyperglycemia and atherosclerotic plaque inflammatory markers. The degree of FoxO1 acetylation results in more complex inflammatory state. In conclusion, our study suggests that FoxO1 acetylation status is physiologically relevant and may contribute to the progression of atherosclerosis in human subjects.

Supported by: Fondazione Roma

## 1192

**Genetic predisposition to dyslipidaemia in an Italian population at risk of type 2 diabetes. The GENFIEV study**

N. Pulizzi<sup>1</sup>, R. Miccoli<sup>1</sup>, C. Bianchi<sup>1</sup>, R. Bonadonna<sup>2</sup>, F. Giorgino<sup>3</sup>, F. Cavalot<sup>4</sup>, G. Cavallo<sup>5</sup>, S. Frontoni<sup>6</sup>, G. Marchesini<sup>7</sup>, L. Groop<sup>8</sup>, S. Del Prato<sup>1</sup>, on behalf of the GENFIEV-FoRiSID Study Group;

<sup>1</sup>Department of Endocrinology and Metabolism, Pisa University, Italy,

<sup>2</sup>Department of Medicine, University of Verona, Italy, <sup>3</sup>Department

of Emergency and Organ Transplantation, University of Bari, Italy,

<sup>4</sup>Department of Clinical Biological Sciences, University of Turin, Italy,

<sup>5</sup>Department of Clinic and Medical Therapy, University of Rome "La

Sapienza", Italy, <sup>6</sup>Department of Internal Medicine, University of Rome Tor

Vergata, Rome, Italy, <sup>7</sup>Clinical Dietetics, Alma Mater Studiorum University

of Bologna, Italy, <sup>8</sup>Department of Endocrinology, University of Lund, Malmö, Sweden.

**Background and aims:** GWAS have shown new genetic variants to be associated with lipid traits. We evaluated these associations in the GENFIEV (GENetics, pathoPHYSiology and EVolution of type 2 diabetes) study, a multicenter nationwide Italian study designed to recruit individuals at risk of developing T2DM, whose clinical characteristics and lipid profile were measured.

**Materials and Methods:** In 864 subjects (56.6% women; age 49.5±11.2 yrs; BMI 29.1±5.3 Kg/m<sup>2</sup>) we performed an OGTT with measurement of C-peptide and fasting insulin. Insulin resistance was assessed by HOMA-IR, while beta-cell function was estimated by the Insulinogenic Index and minimal model analysis of plasma glucose and C-peptide. We measured lipid concentrations according to standard enzymatic methods. We genotyped 19 variants. Obesity (BMI≥30 kg/m<sup>2</sup>) was present in 40% and type 2 diabetes in 14.2%. Associations with lipid profile were investigated by linear regression while logistic regression was used to explore associations with MS and obesity. All analyses were adjusted for age, gender, BMI and type 2 diabetes, appropriately.

**Results:** As described in other populations, we found that in individuals at risk of diabetes rs1260326 (GCKR), rs780094 (GCKR) and rs17145738 (BCL7B,TBL2,MLXIPL) were significantly (p=0.05 or less) associated with increased triglycerides; rs12654264 (HMGCR) and rs780094 (GCKR) with high LDL-Cholesterol (C); rs4846914 (GALNT2) and rs1800775 (CETP) with low HDL-C; rs328 (LPL) with low triglycerides; rs1800588 (LIPC), rs328 (LPL) with high HDL-C. Rs1801282 (PPARG) was associated with high HOMA-IR; rs8050136 (FTO) with obesity; rs1260326 and rs780094 with MS. Moreover, new associations were found between rs1260326 (p=0.005) and high LDL-C; rs1260326 (p=0.0002), rs12654264 (p=0.01), rs17145738 (p=0.03), rs1800588 (p=0.01), rs3890183 (p=0.04) and rs780094 (p=0.0002) with increased Total-C and rs2156552 (p=0.03) with higher triglycerides.

**Conclusion:** While we confirm in our Italian cohort at risk for type 2 diabetes the association between lipid profile with some of the genetic variants previously identified by GWAS, new associations have been found as well. These observations need to be confirmed in other populations as well, and, even more importantly, it will be critical to assess whether causal alleles at each locus affect risk for cardiovascular disease.

*Clinical Trial Registration Number: NCT00879801*

*Supported by: FoRiSID, Rome, Italy with an unconditioned grant from Eli Lilly, Italy*

## 1193

**Genome wide expression profiling uncovers molecular mechanisms of diabetic cardiomyopathy**

J. Lasheras<sup>1</sup>, M. Pujals<sup>1</sup>, J.E. Feliu<sup>2</sup>, J.A. Villena<sup>1,3</sup>;

<sup>1</sup>Vall d'Hebron-Research Institute, <sup>2</sup>Universidad de Castilla-La Mancha,

<sup>3</sup>CIBERDEM, Barcelona, Spain.

**Background and aims:** Cardiovascular disease is the major cause of mortality in diabetic patients. Risk factors such as hypertension and coronary artery disease contribute to the high prevalence of cardiovascular dysfunction in diabetic population. However, pathological and clinical studies have shown that diabetes is associated with impaired cardiac contractility independently of atherosclerosis or hypertension, a condition that is defined as diabetic cardiomyopathy. The disease is characterized by decreased diastolic function and reduced left ventricle ejection time. These functional alterations are accompanied by structural changes, including left ventricle hypertrophy and fibrosis in the advanced stages of the disease. At the cellular level, diabetic hearts present an abnormal oxidative metabolism characterized by the absolute reliance on

fatty acids as the main source for ATP production and a decrease in glucose utilization. Still, the underlying mechanisms of diabetic cardiomyopathy have not been unraveled. Here, we have undertaken a genome wide expression profiling study in hearts of diabetic mice to uncover the genetic pathways that lie beneath the development of diabetic cardiomyopathy.

**Materials and methods:** To study the mechanisms underlying the development of diabetic cardiomyopathy, we have performed a gene expression profile study in hearts of 12-week old diabetic db/db mice using DNA microarrays.

**Results:** At 12 weeks of age, db/db mice are severely diabetic, exhibiting fasting hyperglycemia and hyperinsulinemia. Associated to decreased cardiac contractility, hearts of diabetic mice exhibited a 15 % decreased in ventricular mass. Paradoxically, despite a decreased ventricular weight, hearts from diabetic mice showed cardiomyocyte hypertrophy. However, measurement of total genomic DNA content revealed a 20 % reduction in cell number in hearts of db/db mice. Such decrease in cellularity could be attributed to an increase in cell apoptosis, since diabetic hearts exhibited an increased number of caspase-3-positive cells. Genome wide expression profiling using DNA microarrays revealed that nearly 600 genes (P<0.01) were significantly altered in diabetic hearts. Gene enrichment analysis showed that most of down-regulated genes corresponded to genes associated with the immune function, although a decrease in genes involved in the regulation of apoptosis or glycolysis was also found. Among the up-regulated genes, a notable enrichment in genes related to fatty acid, ketone bodies and amino acid metabolism was found. Also, an increased expression of mitochondrial genes and genes involved in the contractile function of heart was observed. A screening for potential regulators of gene expression among the differentially expressed genes, found that members of the PPAR, PGC-1 and ERR families of transcriptional regulators were dysregulated in diabetic hearts.

**Conclusion:** Our results suggest that an alteration in the expression of transcriptional regulators of the PPAR, ERR and PGC-1 families could underlie the cellular and metabolic derangements observed in hearts of diabetic db/db mice.

*Supported by: Marató de TV3, Grant #082610 to J.A.V.*

## 1194

**Candidate genes for Alzheimer's disease in patients with type 2 diabetes mellitus**

M. Vankova, P. Lukasova, D. Vejrazkova, O. Bradnova, K. Dvorakova,

J. Vcelak, B. Bendlova;

Department of molecular endocrinology, Institute of Endocrinology, Prague, Czech Republic.

**Background and aims:** Many studies have revealed that type 2 diabetes mellitus (T2DM) is a risk factor for cognitive dysfunction or dementia, especially those related to Alzheimer's disease (AD). The aim of the presented study was to compare the frequencies of polymorphisms of candidate genes for AD in the sets of T2DM patients and non-diabetic subjects and to evaluate beta cell secretion, insulin sensitivity and lipid levels of the study subjects in relationship to the described polymorphisms.

**Materials and methods:** The study comprised of 1124 subjects. Detailed biochemical and anthropometric characteristics of 368 T2DM patients and 756 non-diabetic subjects were collected. Except of T2DM patients, oral glucose tolerance test with sampling for blood glucose, insulin and C-peptide was performed. Selected polymorphisms of candidate genes for Alzheimer's disease (APOE rs429358, rs7412, TOMM40 rs2075650, rs157580, rs8106922, PICALM rs3851179, GLP1R rs6923761, rs1042044) were determined by ABI TaqMan SNP Genotyping Assays. Statistics was done using NCSS 2004 (chi-square, Mann-Whitney, ANOVA).

**Results:** The genotypic distribution was significantly different between T2DM and non-diabetics only in PICALM gene (p<0.01). In non-diabetics, the homozygotes for minor allele in comparison to the homozygotes for common allele had higher insulin secretion (HOMA-F; p<0.05) and they had also lower insulin sensitivity (OGIS; p<0.05). There was no difference in lipid spectra between minor and common homozygotes in both sets.

**Conclusion:** Our study suggests that the AD risk allele of the polymorphism rs3851179 in the PICALM gene is more frequent in T2DM patients in comparison to non-diabetics. PICALM gene encoding phosphatidylinositol binding clathrin assembly protein, which is involved in AP2-dependent clathrin-mediated endocytosis at the neuromuscular junction, could be important also in the glucose metabolism.

*Supported by: IGA MHCZ NT/13543-4*

## 1195

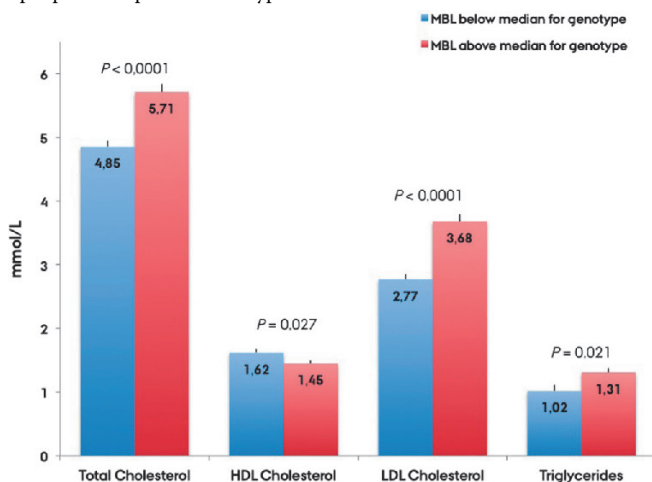
**Association between plasma lipids and mannose-binding lectin (MBL) genotype and circulating MBL levels in patients with type 1 diabetes**T.K. Hansen<sup>1,2</sup>, P. Høyem<sup>1,2</sup>, S. Thiel<sup>3</sup>, P. Rossing<sup>4,2</sup>, A. Flyvbjerg<sup>1,2</sup>, L. Tarnow<sup>4,2</sup><sup>1</sup>Department of Endocrinology and Internal Medicine, Aarhus University Hospital, <sup>2</sup>Department of Clinical Medicine, Aarhus University, <sup>3</sup>Department of Biomedicine, Aarhus University, <sup>4</sup>Steno Diabetes Center, Gentofte, Denmark.

**Background and aims:** Mannose-binding lectin (MBL) may activate the complement system upon binding to glycosylated proteins. MBL levels are elevated in patients with type 1 diabetes, and high MBL genotypes and serum concentrations are associated with susceptibility to micro- and macrovascular complications. Recent data suggest a role of MBL in lipid metabolism, and the aim of the present study was to investigate the association between MBL and circulating lipid levels in statin-naïve patients with type 1 diabetes.

**Materials and methods:** MBL genotypes and serum concentrations of MBL, total-, HDL-, LDL-cholesterol, and triglycerides were determined in an established cohort of 391 patients with type 1 diabetes (199 with diabetic nephropathy, 192 with persistent normoalbuminuria). None of the patients were taking lipid-lowering drugs at the time of sample collections.

**Results:** The mean age of the patients was 41.8±9.9 years, and the average duration of diabetes 27.2 ± 8.2 years. Polymorphisms in the MBL gene associated with low MBL levels were detected in 183 patients. There were no differences in triglycerides, total-, HDL- and LDL-levels between patients with high and low MBL genotypes. Among the 208 patients with high MBL genotypes, however, there was a strong positive correlation between MBL levels and total cholesterol ( $r=0.36$ ,  $P<0.0001$ ), LDL-cholesterol ( $r=0.41$ ,  $P<0.0001$ ) and triglycerides ( $r=0.28$ ,  $P<0.0001$ ) and a significant negative correlation to HDL-cholesterol levels ( $r=-0.20$ ,  $P=0.003$ ). When patients with high MBL genotypes were divided according to the median MBL level for the group, triglycerides, total- and LDL-cholesterol levels were significantly higher among patients with high MBL levels, while HDL-cholesterol levels were significantly lower as can be appreciated from the figure.

**Conclusion:** Plasma lipids are unaffected by MBL genotype, but there may be a common pathway leading to increased MBL levels and an unfavourable lipid profile in patients with type 1 diabetes.



## 1196

**The 9p21 myocardial infarction risk locus does not associate with peripheral artery disease in patients with type 2 diabetes mellitus**E. Russo<sup>1</sup>, D. Lucchesi<sup>1</sup>, L. Pucci<sup>2</sup>, S. De Cosmo<sup>3</sup>, M. Garofolo<sup>1</sup>, R. Miccoli<sup>1</sup>, V. Trischitta<sup>3</sup>, G. Penno<sup>1</sup>, S. Del Prato<sup>1</sup><sup>1</sup>Endocrinology and Metabolism, University of Pisa, <sup>2</sup>IBBA, CNR Pisa,<sup>3</sup>IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy.

**Background and aims:** The chromosome 9p21 (Chr9p21) locus can be considered today the most robust genetic markers for coronary artery disease (CHD). The association between this locus and CHD has been replicated several times. Subsequent work showed associations of Chr9p21 with a number of other cardiovascular disease traits, such as carotid artery plaque, stroke, aneurysms, heart failure and cardiovascular mortality, suggesting a more gen-

eral role in vascular biology. Importantly, Chr9p21 lacks associations with common cardiovascular risk factors, such as lipids and hypertension indicating that the locus exerts its effects through largely independent mechanisms. Recently, genome-wide association studies identified a significant association on Chr9p21 with ankle-brachial index (ABI), a noninvasive measure of peripheral arterial disease (PAD).

**Materials and methods:** It was the aim of our study to investigate whether the CAD locus on Chr9p21 (as represented by SNP rs2383206) is associated with continuous ABI and PAD (ABI<0.9) in 937 unrelated type 2 diabetic patients of European ancestry (550 males, 58.7%; 387 females, 41.3%). Typing was performed by means of TaqMan assay implemented on an HT7900 Applied Biosystems platform. Linear and logistic regression models were used to test the SNP for association with ABI and PAD.

**Results:** Patients were 59.2±7.5 years old with a diabetes duration (DD) of 10.4±9.0 years (BMI 29.4±5.4 kg/m<sup>2</sup>; sBP 142±19 mmHg, dBP 82±10 mmHg). HbA1c was 7.7±2.7% (median value 7.45%; first and third quartile thresholds 6.75 and 8.25%, respectively). Genotypes distribution was: AA (n. 146) 15.6%; GA (n. 452) 48.2%; GG (n. 339) 36.2%; 128 patients (13.7%) had PAD. The sample was in Hardy-Weinberg equilibrium as investigated by the chi-square test. Among genotypes have not been observed differences as far as age, DD, smoking, BMI, SBP, DBP, HbA1c, total-, LDL- and HDL-cholesterol, triglycerides, uric acid, fibrinogen, urinary A/C ratio and eGFR by MDRD. No association was found between the rs2383206 genotype and ABI considered as a continuous trait (AA: ; GA: ; GG: ; ANOVA oneway,  $p=$ ). Furthermore, no association was found between rs2383206 and PAD prevalence (AA 15.1%; GA 13.1%; GG 13.9%; chi-square 0.399,  $p=0.819$ ). No interaction was observed between rs2383206 and poor glycemic control defined as an HbA1c above the median or within the fourth quartile. No differences were observed between genders or in subjects with compared to those without family history of CHD. In a logistic stepwise regression PAD was associated with age, sBP, smoking ( $p<0.001$  for all), dBP, male gender ( $p=0.001$  for both) and A/C ratio ( $p=0.035$ ) (model 1) or CHD ( $p<0.001$ ) (model 2), but not to rs2383206 or HbA1c.

**Conclusion:** In our cohort of type 2 diabetes patients, the rs2383206 SNP, previously related to the CAD risk mainly in presence of poor glycemic control, does not associate with continuous ABI and PAD phenotypes.



## PS 105 Epidemiology and cardiovascular complications

1197

### Predictors of ischaemic heart disease and cerebrovascular attack in late elderly diabetic individuals: the roles of HDL-cholesterol and the LDL-C/HDL-C ratio

T. Hayashi<sup>1</sup>, H. Itoh<sup>2</sup>, A. Araki<sup>2</sup>, H. Sone<sup>3</sup>, H. Watanabe<sup>4</sup>, T. Ohru<sup>5</sup>, K. Yokote<sup>6</sup>, M. Takemoto<sup>6</sup>, M. Noda<sup>7</sup>, K. Ina<sup>1</sup>, H. Nomura<sup>1</sup>, Japan CDM Investigators group;

<sup>1</sup>Department of Geriatrics, Nagoya University Graduate School of Medicine,

<sup>2</sup>Division of Diabetes, Metabolism and Endocrinology, Tokyo Metropolitan

Geriatric Hospital, <sup>3</sup>Department of Internal Medicine, Endocrinology

and Metabolism, Graduate School of Comprehensive Human Sciences,

University of Tsukuba, <sup>4</sup>Department of Clinical Pharmacology and

Therapeutics, Hamamatsu University School of Medicine, <sup>5</sup>Department of

Geriatrics, Tohoku University School of Medicine, Nagoya, <sup>6</sup>Division of

Diabetes, Metabolism and Endocrinology, Department of Internal Medicine,

Chiba University School of Medicine, Chiba, <sup>7</sup>Department of Diabetes

and Metabolic Medicine, National Center for Global Health and Medicine, Tokyo, Japan.

**Background and aims:** Dyslipidemia, especially LDL cholesterol (LDL-C), is a risk factor for ischemic heart disease (IHD) in middle-aged diabetic individuals. However, the factors predicting IHD and a cerebrovascular attack (CVA) in older patients are not well known.

**Materials and methods:** This study was designed as a prospective cohort study (Japan Cholesterol and Diabetes Mellitus Study) with 4,014 type 2 diabetic patients (1,936 women; mean age 67.4±9.5 years, median 70 years, n=126175 years; disease duration 9.6±8.0 years). The levels of lipids, glucose, and other factors such as blood pressure were investigated relative to IHD or a CVA using the multivariate Cox hazard model.

**Results:** One hundred fifty-three cases of IHD and 104 CVAs (7.8 and 5.7/1000 people per year, respectively) occurred over 5.5 years. A lower HDL cholesterol (HDL-C) level and the female gender were correlated with IHD in patients ≥75 years old (hazard ratio (HR): 0.629, P<0.01, 1.132, P<0.05), in contrast, a higher systolic blood pressure (SBP), hemoglobin A1C (HbA1C) and LDL-C were correlated with IHD in subjects <65 years old (P<0.05). In all subjects, a higher LDL-C/HDL-C ratio was correlated with IHD. HDL-C levels were also correlated with CVAs in patients ≥65 years old (OR: 0.536, P<0.01). Kaplan-Meier estimator curves showed that IHD occurred more frequently in patients <65 years old in the highest quartile for LDL-C/HDL-C ratio and in patients ≥75 years old in the lowest quartile for HDL-C. CVAs were also most frequent in patients ≥75 years old in the lowest quartile for HDL-C.

**Conclusion:** IHD and CVAs in late elderly diabetic patients were predicted by the HDL-C level. The LDL-C and HbA1C levels and SBP are risk factors for IHD in non-elderly individuals, and LDL-C/HDL-C may represent the effects of both the LDL-C and HDL-C levels. These age-dependent differences in risk are important for generating an individualized strategy to prevent atherosclerotic disease.

Clinical Trial Number: UMIN00000516

Supported by: Japanese Ministry of Health, Welfare and Labor

1198

### Relationship between cardiovascular risk factors and adipokines in patients with diabetes and chronic hepatitis C

D. Ramona Maria<sup>1</sup>, R. Emilia<sup>1,2</sup>, P. Cristina<sup>1</sup>, N. Raluca<sup>1</sup>, E. Georgiana<sup>1</sup>, R. Florin<sup>3</sup>, R. Gabriela<sup>1,2</sup>;

<sup>1</sup>INDNBM N. Paulescu, <sup>2</sup>University of Medicine and Pharmacy Carol Davila,

<sup>3</sup>Emergency Military Hospital Carol Davila, Bucharest, Romania.

**Background and aims:** Of the study were to evaluate the associations between chronic hepatitis C (CHC), insulin resistance (IR), cytokines and cardiovascular risk, in patients with CHC and type 2 diabetes.

**Materials and methods:** Observational, multicenter study that included 113 patients with CHC and diabetes. There were followed anthropometric indicators (weight, height, waist circumference, BMI (body mass index)). Biochemical parameters followed were blood glucose, glycosylated hemoglobin, lipid profile (cholesterol, triglyceride, HDL-cholesterol), liver profile (ALT, AST,

GGT, bilirubin, albumin, total protein), blood count, cytokines (adiponectin, leptin, TNF-α, IL-6, resistin). IR was determined using Homeostasis model assessment (HOMA-IR). The liver fibrosis was non-invasively assessed using the Forns index; a value < 4.2 excludes liver fibrosis and a value > 6.9 is a predictor for significant fibrosis. The 10-year coronary heart disease (CHD) was calculated for each patient using the UKPDS risk engine.

**Results:** The average age of the evaluated patients was 53.3±7.8 years, 46% females (n=52), with type 2 diabetes for 2.3 years. Metabolic syndrome (MetS) was present in 88 patients (77.9%). Median serum concentrations of leptin, TNF-α, IL-6, resistin were higher in patients with UKPDS-CHD over 30 (all p<0.05). Using the UKPDS score, 8% (n=9) and 45% (n=51) of the patients presented an high and moderate risk for CVD. UKPDS-CHD score was strongly correlated with age (r=0.42, p=0.02), GGT (r=0.41, p=0.03), HOMA-IR (r=0.46, p=0.001), fasting insulinemia (r=0.215, p=0.022), systolic blood pressure (SBP) (r=0.32, p=0.03), TNF-α (r=0.38, p=0.02), IL-6 (r=0.28, p=0.034), cholesterol (r=0.29, p=0.045) and negatively correlated with adiponectin (r=-0.315, p=0.022) and HDL-C (r=-0.37, p=0.001). In this population multiple regression models controlling for BMI indicated that adiponectin (R(2) = 0.16, p=0.034), resistin (R(2) = 0.18, p=0.045), and IL-6 (R(2) = 0.112, p=0.048) were associated with SBP. Forns index >6.9 was associated with increased CHD risk (r=0.341, p=0.0001), HOMA-IR (r=0.47, p=0.001), leptin (r=0.317, p=0.001), TNF-α (r=0.467, p=0.001), IL-6 (r=0.404, p=0.001), resistin (r=0.361, p=0.001), adiponectin (r=-0.352, p=0.001).

**Conclusion:** Increased CHD risk and HOMA-IR are associated with high values of Forns index. Patients with hepatitis C and diabetes have a greater risk to develop cardiovascular diseases. The clinical impact of CHC on cardiovascular risk deserves particular attention because of the growing number of patients with CHC and diabetes. The primary importance of estimating CHD risk is for its use in treatment decision making and patient counseling.

1199

### Atherogenic dyslipidaemia and silent coronary artery disease in patients with type 2 diabetes

P. Valensi<sup>1</sup>, A. Avignon<sup>2</sup>, A. Sultan<sup>2</sup>, B. Chanu<sup>1</sup>, M. Nguyen<sup>1</sup>, E. Cosson<sup>1</sup>;

<sup>1</sup>Department of Endocrinology, Diabetology, Nutrition, Paris Nord

University, Bondy, <sup>2</sup>Department of Diabetology, Lapeyronie hospital, Montpellier, France.

**Objective:** To investigate whether elevated triglycerides and low high-density lipoprotein (HDL) cholesterol (atherogenic dyslipidemia) are predictive of risk for silent myocardial ischemia (SMI) or angiographic coronary artery disease (CAD) in asymptomatic patients with type 2 diabetes.

**Methods:** Cohort study in 1080 asymptomatic patients with type 2 diabetes, a normal 12-lead resting electrocardiogram (ECG), at least one additional cardiovascular risk factor and low density lipoprotein (LDL) cholesterol <3.4 mmol/L. Patients initially underwent screening for SMI by <sup>201</sup>thallium myocardial scintigraphy after an ECG stress test, a pharmacological stress test (dipyridamol injection), or both. Patients with SMI underwent coronary angiography.

**Results:** Overall, 60 (5.5%) patients had atherogenic dyslipidemia (triglycerides ≥2.26 mmol/L and HDL cholesterol ≤0.88 mmol/L). SMI was detected in 292 patients and CAD in 91 patients. CAD was associated with atherogenic dyslipidemia independently of LDL cholesterol levels (p=0.01). In multivariate analyses taking into account the parameters associated in univariate analyses with SMI and then CAD, SMI was associated with atherogenic dyslipidemia (odds ratio 1.8[1.0-3.3]), male gender (OR 2.1[1.5-2.9]), BMI (OR 0.97[0.94-1.00]), retinopathy (OR 1.4[1.1-1.9]), peripheral occlusive arterial disease (POAD: OR 2.5[1.6-3.8]) and mean blood pressure (OR 1.01[1.00-1.03]); CAD was associated with atherogenic dyslipidemia (OR 4.0[1.7-9.2]), male gender (OR 3.0[1.6-5.6]), BMI (OR 0.94[0.90-1.00]), retinopathy (OR 1.7[1.0-2.9]), POAD (OR 4.0[2.1-7.4]) and mean blood pressure (OR 1.03[1.01-1.05]). In the subgroup of 584 patients at LDL cholesterol <2.6 mmol/L, CAD was independently associated with atherogenic dyslipidemia (2.96 [0.97-9.03]; p=0.06).

**Conclusion:** In type 2 diabetic patients including those at LDL cholesterol goal, atherogenic dyslipidemia is associated with an increased risk of silent CAD. Management targeted to this dyslipidemic profile may help to reduce the high residual burden of cardiovascular disease.

## 1200

**Longitudinal changes in serum lipid profiles and their management in representative Australian patients with type 2 diabetes: The Fremantle diabetes study**T.M.E. Davis<sup>1</sup>, K.E. Peters<sup>1</sup>, B.A. Sillars<sup>1</sup>, S.A.P. Chubb<sup>2</sup>, D.G. Bruce<sup>1</sup>, W.A. Davis<sup>1</sup>;<sup>1</sup>School of Medicine and Pharmacology, University of Western Australia,<sup>2</sup>Biochemistry Department, Fremantle Hospital, Fremantle, Australia.

**Background and aims:** Clinical trial evidence of the cardiovascular benefits of statins in type 2 diabetes has changed management substantially, especially over the last 10 years. To examine the magnitude and effects of this change in a real-world setting, we analysed data collected over almost 20 years as part of the Fremantle Diabetes Study (FDS), an observational study of diabetes in an urban Australian community. Our aim was to compare serum lipid profiles and use of lipid-modifying therapies in the 1,296 type 2 participants recruited to Phase 1 (FDS1) between 1993 and 1996 with those of the 1,509 type 2 participants from the same postcode-defined area recruited to Phase 2 (FDS2) between 2008 and 2011.

**Materials and methods:** Comprehensive questionnaire, physical and biochemical data were collected at baseline in both phases. Patients attended after a >10-hour overnight fast with biochemical testing of fasting blood and urine samples performed in a nationally-accredited laboratory using methods that were the same for both Phases or recalibrated when assays changed.

**Results:** FDS2 patients were older than those in FDS1 (mean 65.4±11.7 vs 64.0±11.3 years,  $P=0.001$ ) but a similar proportion was male (51.8 vs 48.6%,  $P=0.10$ ). Statin use was substantially greater in FDS2 (65.9 vs 6.8%,  $P<0.001$ ) in association with a significantly lower serum LDL-cholesterol ( $2.3\pm 0.9$  vs  $3.3\pm 0.9$  mmol/L,  $P<0.001$ ). Serum LDL-cholesterol targets ( $<2.6$  mmol/L in primary prevention,  $<1.8$  mmol/L in secondary prevention) were met by 68.2% of statin-treated FDS2 patients. One third of FDS2 patients eligible for government-subsidised statin therapy (436 of 1,310) remained untreated. Use of fibrates remained low in FDS2 (2.2 vs 3.5%,  $P=0.05$ ).

**Conclusion:** There has been a substantial increase in statin use by Australians with type 2 diabetes over the last two decades. Most statin-treated patients achieve recommended LDL-cholesterol targets but many of those who are eligible for subsidised statin therapy are untreated. A low rate of fibrate use mirrors the comparatively weak evidence base for cardiovascular disease prevention in type 2 diabetes.

Supported by: NHMRC grant 513781

## 1201

**Bone mineral density is reduced in half of Danish insulin dependent type 2 diabetes patients**

T.W. Boesgaard, on behalf of the CIMT Trial Group;

Steno Diabetes Center, Gentofte, Denmark.

**Background and aims:** Prevalence and incidence of low bone mass in patients with type 2 diabetes varies between anatomical region and population. The prevalence of osteoporosis in Denmark is estimated to 20% in women and 10% in men age 55–59 years, increasing to 30% and 15% in 60–64 years, respectively. Both diabetes and osteoporosis are related to bone fractures and vascular complications with increased mortality. Accumulating evidence indicates that the diseases have similar underlying patho-physiological mechanisms. The aim of the present study was to evaluate the prevalence and risk factors for osteopenia and osteoporosis in a Danish trial cohort of patients with type 2 diabetes.

**Materials and methods:** We investigated 429 patients with type 2 diabetes included in the Copenhagen Insulin Metformin Trial (CIMT), recruited from 8 diabetes outpatient clinics in the greater Copenhagen area. Inclusion criteria were age>30 years,  $HbA_{1c}>7.0\%$  and  $eGFR>60$  ml/min. Anthropometric measures, glycaemic status, plasma lipids and creatinine were determined at entry into the trial. Bone Mineral Density (BMD) was measured by Dual Energy X-ray Absorptiometry (DXA). Osteopenia was defined by any T score -1 to -2.5 and osteoporosis as any T score<-2.5. BMD measured in ( $g/cm^2$ ).

**Results:** A total of 429 patients (68% men), age  $61 \pm 9$  years [mean  $\pm$  SD], BMI  $32 \pm 4$  kg/m<sup>2</sup> [mean  $\pm$  SD],  $HbA_{1c}$   $8.5 \pm 1.0\%$  [mean  $\pm$  SD], duration of diabetes 13 years (range 1–40 years), serum creatinine 77 ml/min (range 38–193 ml/min). The prevalence of osteopenia was 43% (46% in men and 38% in women) whereas only 3% (2% of men and 4% of women) had osteoporosis. Patients with osteopenia and osteoporosis were older  $62 \pm 8$  vs  $60 \pm 10$  years,  $p=0.01$ , had lower BMI and lower  $eGFR$ ,  $p<0.03$  than patients with normal

BMD. Patients with elevated serum creatinine had lower femoral hip neck BMD,  $p=0.004$ . Glycaemic control did not differ between groups.

**Conclusion:** Almost half of patients with type 2 diabetes had reduced BMD which is more frequent than expected in age matched non diabetic controls in the Danish population. This was most pronounced in patients with impaired renal function. On the other hand fewer than expected suffered from osteoporosis.

Clinical Trial Registration Number: NCT00657943

Supported by: Novo Nordisk A/S

## 1202

**Socioeconomic status and ischaemic heart disease incidence in people with type 2 diabetes in Scotland**

S. Wild, N. Jones, D. McAllister, The Scottish Diabetes Research Network

Epidemiology Group;

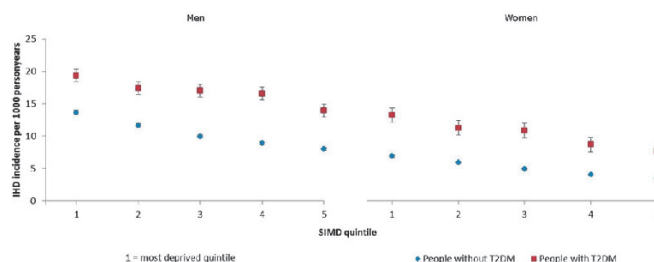
University of Edinburgh, UK.

**Background and aims:** Type 2 diabetes mellitus (T2DM) is more prevalent in people with a lower socioeconomic status (SES) and risk of ischaemic heart disease (IHD) is higher both in people with T2DM and those of low SES. The aim of this study was to investigate whether T2DM and SES increase synergistically the incidence of non-fatal and fatal IHD.

**Materials and methods:** Data were extracted in 2008 from the Scottish Care Information - Diabetes Collaboration dataset and linked to national hospital admissions and mortality data. This analysis is based on the population of people with diagnosed diabetes in Scotland for the period 2005–2007. Data were aggregated by sex, age-group, presence of T2DM, and deprivation quintile (using the area-based Scottish Index of Multiple Deprivation). Poisson regression was used to estimate age and SES adjusted relative risk (RR) for T2DM for incident IHD by sex with comparison to the non-diabetic population and with further comparison to the most deprived quintile to test for interaction. Incident IHD was defined as either a hospital admission or death where I20–25 codes from the tenth revision of the International Classification of Diseases were recorded as primary cause of admission/death or where diabetes was recorded as the underlying cause and IHD listed as a contributing cause of death, when no record of heart disease in the previous ten years exists.

**Results:** Absolute risk of incident IHD was higher in men than women and in people with T2DM than people without diabetes and declined in all groups with decreasing deprivation (Figure). The age and SES-adjusted RR (95% confidence intervals) of IHD were 1.52 (1.47–1.56) for T2DM in men and 1.68 (1.63–1.74) for women. Compared to women who did not have T2DM and were in the most affluent quintile (Q5), relative risks (95% confidence intervals) of incident IHD were 1.31 (1.27–1.35) for women without T2DM in the most deprived quintile (Q1), 2.44 (2.22–2.68) for women with T2DM in Q5, and 3.14 (2.95–3.34) for women with T2DM in Q1 indicating a supra-additive interaction. Similar results were found in men, with RR of 1.33 (1.30–1.37) for men without T2DM in Q1, 2.08 (1.94–2.24) for men with T2DM in Q5 and 2.51 (2.37–2.65) for men with T2DM in Q1 compared to men without T2DM and in Q5.

**Conclusion:** These results indicate that the incidence of non-fatal or fatal IHD in Scotland is strongly related to T2DM status and SES, with evidence of synergy between T2DM and deprivation. These findings suggest that prevention of diabetes could contribute to reducing socio-economic inequalities in IHD incidence.



Supported by: Wellcome Trust/SHIP WTO86113

## 1203

### Temporal trend and geographical variation in hospitalisation for acute diabetic complications in Italy: a nationwide study, 2001–2009

F.L. Lombardo<sup>1</sup>, M. Maggini<sup>1</sup>, G. Bruno<sup>2</sup>;

<sup>1</sup>National Center of Epidemiology, ISS -National Institute of Health, Rome,

<sup>2</sup>Department of Internal Medicine, University of Turin, Italy.

**Background and aims:** Costs of hospitalization for diabetic people accounts for more than 50% of direct costs of the disease. Whereas hospitalizations due to chronic complications depends on long term quality of care, most hospital admissions for acute diabetic complications would have been avoidable with an appropriate outpatient care. In this nationwide study we investigated the temporal trends in hospitalization rates for preventable acute diabetic complications in period 2001–2009 and whereas regional variation within Italy influences this trend.

**Materials and methods:** National Health System data were used to identify 245,500 diabetes-related hospital admissions in Italian people from 2001 to 2009 for acute diabetic complications selecting all records with a primary or secondary discharge diagnosis including either hyperglycemic state: ketoacidosis (ICD-9-CM code: 250.1), hyperosmolar state (ICD-9-CM code: 250.2), other coma (ICD-9-CM code: 250.3), or hypoglycemic coma (ICD-9-CM code: 251.0). Hospitalization rate was calculated as the ratio of the number of hospital discharges and resident population per 100,000, and standardized by age and gender on 2001 Italian population.

**Results:** A mean annual number of 27,300 hospitalizations for acute diabetic complications were recorded in Italy in the period 2001–2009, representing 3.5% of all diabetes-related hospital discharges. Most of hospitalizations (94.4%) were due to hyperglycaemic complications (51.5% for ketoacidosis, 20.4% for hyperosmolarity and 22.5% for other coma) and 5.6% only for hypoglycemic coma. Over the study period, hospitalizations for acute complications decreased in Italy by 39%, from 56.3 per 100,000 in 2001 to 34.5 in 2009 ( $p$  for trend  $<0.001$ ); the decrease was similar for hyperglycaemic and hypoglycaemic complications. The reduction was higher in diabetic people aged 65 years and over, whereas rates were quite stable for those aged 0–39 years. No differences were observed between gender. Large geographical variation were found within Italy, with the lowest rate in the North (26.7 per 100,000 residents in 2009) compared to the Centre (34.1) and the South of Italy (45.4). In spite of similar decrease over time in hospitalization rates, variations across geographic areas remained stable over the study period, with a 3.6-fold difference between the lowest and the highest regional rates in 2009; in particular, an almost ten-fold regional difference in admission rates were found in people aged 0–19 years, which cannot be accounted for by differences in incidence rates of type 1 diabetes.

**Conclusion:** This is the one of the few available study examining hospital admissions for acute complications in diabetic people and the first nationwide study covering the population of Italy over a long time period. Despite hospitalizations for acute diabetic complications decreased by 40% over a nine years period, 22,052 hospital discharges were still registered in 2009. Large geographic variations are evident within Italy, and the amount of this excess cannot be attributed exclusively to regional variability in incidence and prevalence of the disease. The observed 3.6-fold difference is clinically important for a preventable complication with a significant potential for long-term morbidity and mortality. Prevention strategies are needed, particularly in areas identified with the highest rates.

## 1204

### Long-duration diabetes reduces physical function in Chinese adults: Da Qing IGT and diabetes study

Y. An<sup>1</sup>, E. Gregg<sup>2</sup>, P. Zhang<sup>3</sup>, J. Wang<sup>3</sup>, Q. Gong<sup>1</sup>, M. Engelgau<sup>2</sup>, G. Roglic<sup>4</sup>, P.H. Bennett<sup>5</sup>, G. Li<sup>6</sup>;

<sup>1</sup>Endocrine department, Fuwai Hospital, Beijing, China, <sup>2</sup>CDC, Atlanta, USA, <sup>3</sup>DaQing First Hospital, Daqing, China, <sup>4</sup>WHO, Geneva, Switzerland,

<sup>5</sup>NIDDK, Phoenix, USA, <sup>6</sup>China Japan Friendship Hospital, Beijing, China.

**Background and aims:** Diabetes has been shown to reduce physical function by self-report in Chinese adults, but no previous studies have documented such reduction using performance-based measures. This study evaluated physical functional levels measured by performance-based tests among the Chinese elderly with and without diabetes.

**Materials and methods:** In 1986, 630 persons aged 25–74 years with newly diagnosed type 2 diabetes (NDM) were identified by population-based diabetes screening and 519 with normal glucose tolerance (NGT) were selected for

comparison. In 2009, physical function was assessed in 237 (90% of the survivors) from the NDM cohort and 333 (85% of the survivors) from the NGT cohort of whom 230 had not developed diabetes by the time of the 23 year follow-up examination. Physical function was assessed using standardized protocols for grip strength (measured with a hand-dynamometer), balance (tandem stand), walking speed (meters per second), and chair stands (time to stand five times from an armless chair). Individuals were defined as having poor physical function if: 1) their grip strength was below 20th percentile of the population norm, 2) they were unable to balance for 10 secs or more, or 3) their walking speed was less than 0.7m/sec, or 3) they took more than 13.8 secs to complete the chair stands. We compared the physical performance between NDM and those never develop diabetes.

**Results:** Among the people with NDM, 56.1%, 41.9%, 38.4% and 51.9% had poor performance in grip strength, balance, walking speed, and chair stands respectively, compared to 44.0% ( $P<0.01$ ), 19.7% ( $p<0.001$ ), 22.2% ( $p<0.001$ ) and 42.1% ( $p<0.05$ ) of the participants in NGT group. After adjustment for age and sex, participants in the NDM cohort had significantly worse physical function than those in the NGT group (22.7kilogram (kg) force vs. 24.8 ( $p=0.0003$ ) in grip strength; 1.73 vs. 2.43 ( $p<0.001$ ) in balance score; 2.30 vs. 2.77 ( $p=0.0002$ ) in gait speed score. However, the difference in chair stands score between the two cohorts was modest (1.71 vs. 1.93 ( $p=0.05$ )). Participants who did not have diabetes at the end of the study had significantly better physical performance than those with diabetes in all four performance measures.

**Conclusion:** Diabetes significantly reduced many dimensions of objectively measured physical function among elderly Chinese with long duration diabetes.

## 1205

### What are the real risk factors for development of diabetes-related complications in patients with type 2 diabetes?

N. Müller, C. Kloos, G. Wolf, U.A. Müller;

Dept. Internal Medicine III, University Hospital Jena, Germany.

**Background and aims:** To prevent diabetes-related complications a good control of HbA<sub>1c</sub> and blood pressure is commonly accepted to be of major importance. In contrast to this, it is repeatedly reported, that some patients do not acquire complications in spite of off target HbA<sub>1c</sub> and blood pressure, whereas others with good metabolic control develop them. To shed some light on this question we studied the differences in HbA<sub>1c</sub> and blood pressure in patients with type 2 diabetes with and without diabetes-related complications and duration of diabetes of at least 20 years.

**Materials and methods:** We recruited all patients with diabetes mellitus type 2 and time since diagnosis of diabetes of at least 20 years from our electronic patient record EMIL<sup>®</sup>. Data from 296 patients (44 % female, mean age 72.2 years, time since diagnosis of diabetes 27.4 years, BMI 32.7 kg/m<sup>2</sup>, HbA<sub>1c</sub> 6.8 % (51mmol/mol), 91.6% were treated with insulin) were analysed. 9.5% (28) of the patients had no complications, 15.5% (43) had one mild; 9.5% (28) had one severe, 35.1% (104) had two and 31.4% (93) had three diabetes-related complications (retinopathy, nephropathy and neuropathy). We compared patients with no or one mild complication with patients with three complications. The mean follow up was 10.5y. HbA<sub>1c</sub> results were adjusted to the DCCT standard.

**Results:** Patients with no or one mild complication compared to patients with three complications have a lower mean (avg. of the follow up) HbA<sub>1c</sub> by 0.3% (7.4 vs. 7.7% (57 vs. 60.66 mmol/mol);  $p=0.042$ ), a significantly lower mean systolic blood pressure (144 vs. 148 mmHg;  $p=0.015$ ), but a higher diastolic blood pressure (82 vs. 78 mmHg;  $p=0.001$ ). We found no difference between patients with none or one mild complication and patients with three complications in respect of age (70.3 vs. 72.8y;  $p=0.062$ ), time since diagnosis of diabetes (27.3 vs. 28.4y;  $p=0.236$ ), mean BMI (31.5 vs. 32.7 kg/m<sup>2</sup>;  $p=0.236$ ), smoking status (current smokers 6.8 vs. 8.1%;  $p=0.368$ ) and social status (11.3 vs. 11.0;  $p=0.704$ ). In the regression analyses with age, blood pressure, BMI and HbA<sub>1c</sub> as co-variables, we found no significant association of age, BMI and HbA<sub>1c</sub> to the risk of diabetes-related complication. The association for blood pressure was also weak with an OR=1.007 ( $p=0.001$ ) for systolic blood pressure and an OR=0.893 ( $p=0.001$ ) for diastolic blood pressure.

**Conclusion:** In a group of patients with long term diabetes type 2 we found the risk for the development of diabetes-related complications only weakly associated to blood pressure and not associated to HbA<sub>1c</sub>. Those marginal differences to our understanding are unlikely to be the main and only cause for the different susceptibility of the patients in respect to diabetes related



complications. We assume different harmful and hereto unheeded co-factors are responsible for instance an autoimmune reaction or a genetic predisposition.

## 1206

### Does diabetic cardiomyopathy exist? Data from a series of 656 asymptomatic diabetic patients with known cardiac ischemic status

I. Pham<sup>1</sup>, M.-T. Nguyen<sup>2</sup>, I. Banu<sup>2</sup>, S. Chiheb<sup>2</sup>, C. Pillegand<sup>2</sup>, B. Chanu<sup>2</sup>, P. Valensi<sup>2</sup>, E. Cosson<sup>2</sup>;

<sup>1</sup>Explorations Fonctionnelles, <sup>2</sup>Service de diabétologie, AP-HP, HUPSS, Hôpital Jean Verdier, Bondy, France.

**Background and aims:** The aim of the study was to assess the prevalence of sub-clinical cardiomyopathy among patients with type 2 diabetes but without hypertension or coronary artery disease (CAD). Such cardiomyopathy would therefore be due only to diabetes or related metabolic disorders and could actually be named diabetic cardiomyopathy, in the absence of confounding factors.

**Materials and methods:** 656 patients with type 2 diabetes for 14±8 years (359 men, 59.7±8.7 years, HbA1c 8.7±2.1%) and at least one cardiovascular risk factor (hypertension 74%; dyslipidemia 70%; smoking habits 22%; peripheral occlusive arterial disease 10%, nephropathy 39%) but without cardiac symptom, had a cardiac echography at rest; underwent a stress cardiac scintigraphy to screen for silent myocardial ischemia (SMI), and in case of SMI, a coronary angiography to screen for silent CAD.

**Results:** SMI was diagnosed in 206 patients, and 71 of them had silent CAD. In the patients without hypertension or CAD (n=157), left ventricular hypertrophy (LVH: 24.1%) was the most frequent abnormality, followed by left ventricular dilation (8.6%), hypokinesia (5.3%), type 1 abnormal relaxation (4.8%) and systolic dysfunction (3.8%). Neither parameter was associated with LVH nor with left ventricular dilation nor with abnormal relaxation. In multivariate analysis, the parameters associated with hypokinesia were SMI (Odds ratio 14.7 [2.7–81.7], p<0.01) and peripheral occlusive arterial disease (OR 12.2 [1.4–103.1], p<0.05); those associated with systolic dysfunction were SMI (OR 114.6 [1.7–7907], p<0.01), HbA1c (OR 1.9 [1.1–3.2], p<0.05) and body mass index (OR 1.6 [1.1–2.4], p<0.05). LVH was more prevalent among hypertensive patients (hypertension without CAD 34.5%; with CAD 46.7%); and hypokinesia in the patients with CAD (CAD without hypertension 13.3%, with hypertension 13.7%).

**Conclusion:** In asymptomatic type 2 diabetic patients, diabetic cardiomyopathy is highly prevalent and is predominantly characterized by LVH. SMI, obesity and poor glycemic control contribute to systolic dysfunction and/or hypokinesia. Hypertension is associated with more LVH, and CAD with more hypokinesia.

	HTA- SC- n=157	HTA+ SC- n=411	HTA- SC+ n=15	HTA+ SC+ n=56
LVH, (%)	24,1	34,5	30,0	46,7
LV dilation, (%)	8,6	6,5	9,1	12,0
Systolic dysfunction, (%)	3,8	3,5	0	8,7
Hypokinésia, (%)	5,3	8,6	13,3	13,7
Type 1 abnormal relaxation, (%)	4,8	4,4	0	14,3

## PS 106 Microvascular complications in experimental studies

## 1207

### Diabetes-induced liver failure: effects of chronic 1,5-isoquinolinediol and nicotinamide treatment

M. Guzyk, T. Kuchmerovska;

Coenzymes, A.V. Palladin Institute of Biochemistry, Kiev, Ukraine.

**Background and aims:** The liver is known to be abnormally susceptible to oxidative damage from high ambient oxidant levels associated with diabetes. However, the exact mechanism by which oxidative stress could contribute to and accelerate the development of hepatic dysfunction in diabetic mellitus is only partly known and still need to be clarified. Moreover, hyperglycemia as was shown can be linked to cirrhosis and portal hypertension. The present study was designed to investigate the impact of experimental diabetes on the pathophysiological processes development in liver. We also tested whether 1,5-isoquinolinediol (ISO), a recent generation PARP inhibitors and nicotinamide (NAM), as drug with the wide range of metabolic effects can influence liver impairments associated with diabetes in therapeutic doses.

**Materials and methods:** All studies were carried out after 10 weeks of diabetes (streptozotocin, 55 mg/kg of body weight, i. p.) in male Wistar rats treated for 14 days with or without ISO (3 mg/kg, i. p.) and NAM (100 mg/kg, i. p.). The enzyme activities, protein, ascorbate, NAD<sup>+</sup> contents and endogenous antioxidant defence system states were assayed in liver. The activities of caspase 3 (Casp 3), cytochrome P 450 2E1 (CYP 2E1) and content of poly (ADP-ribose) polymerase 1 (PARP-1) were assessed by electrophoresis and immunoblotting.

**Results:** As evidence of oxidative stress and weakening of antioxidant defences in diabetic rats, we established reduction of the liver NAD<sup>+</sup>, substrate of poly ADP-ribosylation, and ascorbate levels by 35.3±4.1 % and 43.2±4.9 %, respectively, vs. control at p<0.05. Notably, that ISO supplementation to diabetic rats had no effect on blood glucose level while NAM caused a slight lowering effect. It was found more than 2.5 fold increases in mean CYP 2E1 expression in diabetic rats and groups treated with ISO as compared to controls. NAM administration was also accompanied with its ability to increase expression but to a less extent, p<0.05. It is not excluded, that exacerbation in mean CYP 2E1 expression in the livers of diabetic rats and animals treated with ISO and NAM may be associated directly or indirectly with elevated plasma ketone levels. We observed that caspase-3 was activated in the liver homogenates all groups animals as compared to control as result of PARP-1 cleavage. Administration of NAM partially inhibited Casp 3 activation. The SOD activity in diabetic rats was slightly increased as compared to control group (p<0.05). ISO and NAM exerted normalizing effects on this parameter.

**Conclusion:** Our findings indicate that PARP-1 inhibitors, such as ISO and NAM may be involved in improvements liver functions not only PARP-1 inhibition but also through multiple mechanisms including apoptosis. In conclusion, we suggest that these compounds might be useful agents for treating liver failures related to diabetes.

## 1208

### Metabolic deviations and liver injury in two diabetes mellitus models

J. Sokolovska<sup>1,2</sup>, S. Isajevs<sup>2</sup>, E. Rostoka<sup>2</sup>, L. Baumane<sup>1</sup>, J. Sharipova<sup>1</sup>,

O. Sugoka<sup>3</sup>, D. Isajeva<sup>2</sup>, I. Kalvinsh<sup>1</sup>, N. Sjakste<sup>1,2</sup>;

<sup>1</sup>Latvian Institute of Organic Synthesis, <sup>2</sup>Faculty of Medicine, University of Latvia, Riga, <sup>3</sup>Institute of Biology, University of Latvia, Salaspils, Latvia.

**Background and aims:** Fatty liver and non-alcoholic fatty liver disease (NAFLD) are implicated in pathogenesis of the insulin resistance and diabetes mellitus (DM). Glucotoxicity caused by chronic hyperglycaemia, as well as hyperlipidaemia have been shown to initiate accumulation of fat in the liver. Further, inflammation in the fatty liver might be mediated by increased iNOS expression followed by increased nitric oxide (NO) concentration. Changes in GLUT1 expression have been shown to be involved in development of some diabetic complications, however data about its role in NAFLD are scarce. In this work, we have studied changes in GLUT1, NO concentration and histological activity index (HAI) in two rat experimental models of DM: severely hyperglycaemic streptozotocin model (STZ model) and moderately hyperglycaemic hyperlipidaemic model, induced by high fat diet and low dose streptozotocin injections (HS model).

**Materials and methods:** Wistar rats aged 2 months were used. Rat STZ model was induced by single streptozotocin injection 50 mg/kg *i/v*. HS model was induced by feeding rats high fat diet for 2 weeks followed by 30 mg/kg *i/v* streptozotocin injection. 6 weeks after induction of both models of diabetes, GLUT1 mRNA and protein expression in liver were studied by means of real time RT-PCR and immunohistochemistry correspondingly. Production of NO was monitored by means of ESR spectroscopy of Fe-DETC-NO complex. Fibrotic, inflammatory and necrotic changes as well as fatty infiltration of the liver were expressed as HAI, as previously described.

**Results:** Mean non-fasting plasma glucose concentrations in the groups were: control  $5.48 \pm 0.45$  mmol/l, STZ  $58.11 \pm 5.53$  mmol/l, HS  $18.66 \pm 0.82$  mmol/l. In STZ group, plasma lipids were in similar concentrations as in control group, increased triglycerides and total cholesterol concentration were observed in the HS group (triglycerides: control  $1.46 \pm 0.09$  mmol/l, HS  $3.46 \pm 0.37$  mmol/l,  $p < 0.05$ ; total cholesterol: control  $1.60 \pm 0.04$  mmol/l, HS  $3.34 \pm 0.41$  mmol/l,  $p < 0.05$ ). Similarly, NO concentration was increased statistically significantly ( $p < 0.05$ ) in the liver of rats with severe hyperglycaemia (STZ group), but it did not change in HS rats (control  $36.8 \pm 10.3$  ng/g tissue versus STZ group  $142.1 \pm 31.1$  ng/g tissue,  $p < 0.05$ ; HS group  $35.4 \pm 9.8$  ng/g). Liver HAI was increased in STZ group,  $8.6 \pm 0.107$  versus control,  $2.60 \pm 0.74$ ,  $p = 0.008$ . In HS group, some insignificant increase in fatty degeneration was observed. GLUT1 protein expression was highly elevated in STZ group,  $16 \pm 3$  GLUT1 positive cells/mm<sup>2</sup> versus control,  $5 \pm 2$  GLUT1 positive cells/mm<sup>2</sup>,  $p = 0.007$ , but it did not change in HS group.

**Conclusion:** These results show that histologic changes similar to those observed in NAFLD in rat models of DM are caused by severe hyperglycaemia but not hyperlipidaemia, and are associated with increased NO production in the liver. Increased GLUT1 transporter expression might be involved in toxic effects of glucose in the liver.

*Supported by: ESF project «Support for Doctoral Studies at University of Latvia»*

## 1209

### Podocyte-specific angiotensin-1 overexpression ameliorates diabetes-induced albuminuria: implication of VEGF-A/VEGF receptor system

L. Gnudi<sup>1</sup>, D.A. Long<sup>2</sup>, K.E. White<sup>3</sup>, J. Pan<sup>1</sup>, M. Locatelli<sup>1</sup>, S.J. Smillie<sup>1</sup>, M. Kolatsi-Joannou<sup>2</sup>, A. Hayward<sup>1</sup>, A.S. Woolf<sup>4</sup>, C. Dessapt-Baradez<sup>1</sup>; <sup>1</sup>Cardiovascular Division, King's College London, <sup>2</sup>Nephro-Urology Unit, University College London, <sup>3</sup>Medical School, University of Newcastle, Newcastle upon Tyne, <sup>4</sup>Developmental and Regenerative Medicine Research Group, University of Manchester, London, UK.

**Background and aims:** Diabetic nephropathy (DN) is the leading cause of end-stage renal failure. Proteinuria is an early feature of DN and a strong predictor of disease progression, and activation of VEGF signalling pathway has been implicated in its pathogenesis. Diabetic glomerulopathy is characterised by increased angiotensin (Ang)-2 and reduced Ang-1 expression levels. Ang-1 activates Tie-2 receptor leading to endothelial cell survival and vessel integrity, while Ang-2 acts as an antagonist. Glomerular Ang-2 overexpression increases albuminuria in mice. We hypothesise that increased levels of Ang-2 in the diabetic glomeruli is an important player in the early stages of DN. Therefore by glomerular overexpression of Ang-1, and thus decreasing the Ang-2/Ang-1 ratio, we expect an amelioration of early diabetic glomerulopathy.

**Materials and methods:** We generated transgenic (TG) mice with podocyte-specific doxycycline (DOX)-inducible overexpression of Ang-1. Five weeks old male TG and non transgenic (nTG) mice were made diabetic with daily streptozotocin injections for 5 days. Non-diabetic (ND) and diabetic (D) mice were randomised to receive either DOX or vehicle (VEH: 5% sucrose) alone, in drinking water starting at 8 weeks of age for 10 weeks. Blood pressure and urinary albumin were measured at the end of the study, and tissues collected and processed for molecular determinations, and glomerular ultrastructural morphology analysis by electron microscopy.

**Results:** Diabetes was characterised by an increase in Ang-2/Ang-1 ratio and by an increase in albuminuria in D nTG VEH or DOX-treated mice (D-nTG vs ND-nTG,  $p < 0.05$ ). In D TG mice, the DOX-mediated increase in Ang-1 expression, resulted in decrease of the Ang-2/Ang-1 ratio and in a 40% reduction in albuminuria when compared to D nTG DOX-treated mice (D-TG-DOX vs D-nTG-DOX,  $p < 0.05$ ). Nephron expression levels were reduced in D animals ( $p < 0.05$ ), but no difference was observed between nTG and TG DOX-treated D mice. Podocyte-specific Ang-1 overexpression had no effect on albuminuria in ND mice. Diabetes was paralleled by kidney hypertrophy, mesangial expansion and thickening of the glomerular basement membrane

(D-nTG vs ND-nTG,  $p < 0.05$ ), but podocyte-specific Ang-1 overexpression did not ameliorate these parameters. Systolic blood pressure was raised in D mice but no difference was observed between TG and nTG DOX-treated D mice. Kidney cortex VEGF protein expression and VEGF receptor-2 phosphorylation were increased, while soluble VEGF receptor-1 (sFlt-1) was decreased in D versus ND mice (D-nTG vs ND-TG,  $p < 0.05$  for all). In D TG mice, overexpression of Ang-1 was paralleled by sFlt-1 upregulation and reduction in VEGF receptor-2 phosphorylation (D-TG DOX vs D-nTG DOX,  $p < 0.05$ ).

**Conclusion:** Podocyte-specific overexpression of Ang-1 reverse the diabetes-mediated decrease in Ang-1/Ang-2 ratio and ameliorates albuminuria accompanied by upregulation of sFlt-1 and inhibition of the VEGF-A/VEGF receptor signalling system. No effects of Ang-1 overexpression were observed in diabetes-mediated glomerular ultrastructural changes.

*Supported by: DUK, KRUK, EFSD Clinical Research Grant*

## 1210

### Diabetes-induced changes in the extracellular matrix of the kidney and the effect of the Cu (II) chelator TETA

S. Brings<sup>1</sup>, S. Zhang<sup>1</sup>, B.Y. Chong<sup>1</sup>, D. Gong<sup>1</sup>, G.J.S. Cooper<sup>1,2</sup>; <sup>1</sup>School of Biological Sciences, University of Auckland, New Zealand, <sup>2</sup>Biomedical Research Centre, University of Manchester, UK.

**Background and aims:** Our group has shown that treatment with triethylenetetramine (TETA), a Cu (II) chelator, improves the function of the kidney and heart in diabetic humans and rats. One potential mechanism of action of TETA is the inhibition of oxidative stress in diabetes which can lead to non-enzymatic post-translational modifications (PTMs) of proteins. Extracellular matrix (ECM) proteins like collagen are particularly prone to the formation of PTMs due to the slow turnover rate. Thus structural and functional changes of the renal ECM in TETA- or placebo-treated diabetic and control animals were investigated, with a focus on PTMs of collagen.

**Materials and methods:** Male Wistar rats were injected with STZ (55 mg/kg, *i.v.*), to induce diabetes, or saline (for controls). TETA treatment (20 mg/day) was started 8 weeks after injection in half of the STZ- and saline-injected rats (4 groups,  $n = 12$  per group) and maintained for 8 weeks, after which the kidneys were collected. Kidney collagen was extracted and analysed for PTMs. Collagen content of renal cortex was determined by hydroxyproline measurement. Relative mRNA levels of collagen and related genes were measured via RT-qPCR. Relative protein levels were determined by western blotting. Statistical analysis was performed by two way ANOVA followed by Tukey-Kramer post-hoc test or Mann-Whitney U-test for non-parametric data.

**Results:** Messenger RNA levels for several isoforms of collagen were decreased in diabetes (Col1a2,  $p = 0.013$ ; Col4a4,  $p < 0.0001$ ; Col4a5,  $p = 0.004$ ; Col6a1,  $p = 0.014$ ; Col6a2,  $p = 0.008$ ; Col6a3,  $p = 0.035$ ) accompanied by a decrease in collagen protein levels ( $p = 0.003$ ). TETA treatment restored collagen protein levels to normal ( $p = 0.012$  STZ/placebo vs STZ/TETA) with no effect on mRNA levels. Protein levels of the collagen-degrading enzyme cathepsin L (CtsL) were increased in diabetes ( $p = 0.002$  and  $p = 0.0005$  for double chain and single chain respectively) along with mRNA levels ( $p = 0.013$ ). Pepsin digestibility of collagen extracts was decreased in diabetes ( $p = 0.045$ ). Collagen cross-linking enzyme lysyl oxidase (Lox) mRNA levels ( $p < 0.0001$ ) as well as protein levels ( $p = 0.017$ ) were increased. TETA had no effect on these parameters. The advanced glycation end-product (AGE) carboxymethyllysine (CML) was 4.2-fold more abundant in collagen extracts from diabetic kidneys ( $p = 0.014$ ) while the increase in extracts from TETA treated diabetic kidneys was only 2.0-fold (STZ/TETA group not significantly different from Sham/placebo).

**Conclusion:** Protein levels of collagen may be lower in diabetic kidneys due to the decreased mRNA synthesis. Increased CtsL mRNA and protein levels in diabetes can contribute further to the decreased collagen protein levels. Decreased pepsin digestibility of collagen in diabetes can be explained by increased Lox protein levels or enhanced AGE (CML) formation. Collagen protein levels were found to be lower in diabetes despite decreased pepsin digestibility of collagen, enhanced AGE formation and increased Lox protein level. Thus the correlation between AGE formation, *in vitro* pepsin digestibility and collagen accumulation in the diabetic kidney is not straightforward due to the effect of proteases like CtsL and transcriptional regulation. TETA treatment may normalise collagen levels in diabetic kidneys via inhibition of cathepsin L activity and/or the decrease of AGE-formation.

*Supported by: HRC, New Zealand*

## 1211

### Exogenous and endogenous hydrogen sulfide protects endothelial cells from methylglyoxal-mediated damage

N.J. Murphy<sup>1</sup>, J.G. Mabley<sup>2</sup>;

<sup>1</sup>Brighton and Sussex Medical School, Hove, <sup>2</sup>School of Pharmacy & Biomolecular Sciences, University of Brighton, UK.

**Background and aims:** Methylglyoxal (MGO), a glycolysis derived reactive dicarbonyl compound has been implicated as a mediator of diabetic cardiovascular complications. MGO has been shown to cause endothelial cell dysfunction via increased oxidative stress. Hydrogen sulfide is an gaseous transmitter found in the vasculature and synthesized from the amino acid L-cysteine. Hydrogen sulfide has been demonstrated to have a protective role in the vasculature acting as a vasodilator and protecting the endothelium from oxidative stress. The aim of this study is to investigate whether hydrogen sulfide generated exogenously by a chemical donor or endogenously from L-cysteine is able to protect endothelial cell function from MGO-mediated dysfunction.

**Materials and methods:** The effects of MGO on acetylcholine-mediated NO dependent vasorelaxation were measured using ex vivo rat aortic rings exposed to 0.3 mM MGO for 2h, sodium hydrogen sulfide (0.3 and 0.5 mM) or L-cysteine (0.3 and 0.5 mM) was co-administered to investigate any protective effect. In vitro experiments using the EA.Hy926 human endothelial cell line were performed examining the effect of MGO±sodium hydrogen sulfide (0.3 and 0.5 mM) or L-cysteine (0.3 and 0.5 mM) on cell viability (MTT assay) and necrosis/apoptosis levels (propidium iodide/Hoechst staining followed by morphological analysis).

**Results:** MGO (0.3 mM) exposure increased the IC50 for acetylcholine-mediated relaxation from  $0.08 \pm 0.02 \mu\text{M}$  to  $0.42 \pm 0.1 \mu\text{M}$  ( $P < 0.05$ ), with protection being observed with both hydrogen sulfide ( $0.2 \pm 0.04 \mu\text{M}$  and  $0.12 \pm 0.02 \mu\text{M}$ ) and L-cysteine ( $0.13 \pm 0.02 \mu\text{M}$  and  $0.09 \pm 0.03 \mu\text{M}$ ) at 0.3 and 0.5 mM respectively ( $p < 0.05$  vs. MGO alone). Hydrogen sulfide and L-cysteine also protected the endothelial cell line from MGO-mediated loss of cell viability significantly increasing it from  $59 \pm 2\%$  with MGO 0.8 mM alone to  $75 \pm 2\%$  and  $84 \pm 1\%$  with 0.3 and 0.5 mM hydrogen sulfide and  $93 \pm 3\%$  and  $96 \pm 2\%$  with 0.3 and 0.5 mM L-cysteine respectively ( $p < 0.01$  vs. MGO alone). MGO 0.6 mM increased both necrosis (from  $4 \pm 1\%$  to  $20 \pm 5\%$  ( $p < 0.05$  vs. untreated cells)) and apoptosis (from  $1.2 \pm 0.6\%$  to  $7 \pm 2\%$  ( $p < 0.05$  vs. untreated cells)) levels in endothelial cells. Hydrogen sulfide 0.5 mM was again able to significantly protect against the MGO-mediated increase in cell death reducing necrosis to  $9 \pm 3\%$  and apoptosis to  $2 \pm 1\%$  ( $p < 0.05$  vs. MGO 0.6 mM alone). L-Cysteine also protected against MGO-mediated increase in necrosis and apoptosis reducing it to  $7 \pm 2\%$  and  $1.7 \pm 0.3\%$  respectively ( $p < 0.05$  vs. MGO 0.6 mM). Inhibition of the hydrogen sulfide synthesizing enzyme cystathionine- $\gamma$ -lyase with DL-propargylglycine increased the susceptibility of endothelial cells to MGO-mediated loss of cell viability while also partially blocking the protective effect of L-cysteine.

**Conclusion:** Hydrogen sulfide generated either directly from a chemical donor or indirectly from the endogenous substrate L-cysteine provided significant protection against MGO-mediated endothelial cell dysfunction. Hydrogen sulfide also protected the endothelial cell line from the MGO-mediated decrease in cell viability and increase in necrosis and apoptosis. These data suggest that hydrogen sulfide donors or therapies designed to increase endogenous production of hydrogen sulfide by the vasculature may be useful in reducing diabetic cardiovascular complications.

## 1212

### Semaphorin3g regulates endothelial cell fenestration and structure of foot processes

R. Ishibashi<sup>1</sup>, M. Takemoto<sup>2</sup>, Y. Akimoto<sup>2</sup>, S. Onishi<sup>1</sup>, T. Ishikawa<sup>1</sup>, E. Okabe<sup>1</sup>, P. He<sup>1</sup>, K. Kobayashi<sup>1</sup>, M. Fujimoto<sup>1</sup>, H. Kawamura<sup>1</sup>, G. Genov<sup>3</sup>, C. Betsholtz<sup>3</sup>, K. Tryggvason<sup>3</sup>, K. Yokote<sup>1</sup>;

<sup>1</sup>Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan, <sup>2</sup>Department of Anatomy, Kyorin University School of Medicine, Tokyo, Japan, <sup>3</sup>Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Switzerland.

**Background and aims:** Etiologies of diabetic nephropathy are largely unknown. Recent investigations have clarified the potential roles of glomerular podocytes in the development of diabetic nephropathy. The aim of this study is to analyze the functions of Semaphorin3g (Sema3g), which we have recently identified as a podocyte-expressed gene.

**Materials and methods:** Expression patterns of Sema3g mRNA were examined by Northern blot and in situ hybridization. Sema3g was expressed in Cos7 cells, and the proteins in the conditioned medium were analyzed by Western blotting. Sema3g knockout mice (Sema3g KO) were used to determine the function of Sema3g in vivo. Microarray analyses on Affymetrix GeneChip and reverse transcription-PCR (RT-PCR) were performed using isolated glomeruli.

**Results:** Sema3g mRNA was expressed in the kidney, heart, ovary, and at the highest level in the lung. In situ hybridization using embryonic day 18.5 kidneys revealed that Sema3g mRNA was expressed in podocytes at stages from the S-shaped body to mature glomeruli. Sema3g mRNA expression was also observed in arterial endothelial cells outside the glomeruli. The Sema3g gene encodes 782 amino acids consisting of a Sema domain and is classified as a secreted protein. We expressed Sema3g in Cos7 cells and detected approximately 100 kDa protein in the conditioned medium, confirming that Sema3g is a secreted protein. Since the functions of this protein in mammals are unknown, we generated Sema3g KO mice. These mice are viable and exhibit no overt phenotype. Light microscopy revealed that the Sema3g KO mice had no observable glomerular developmental defects. However, electron microscopy revealed thickening of the glomerular basement membrane and effacement of the podocyte foot processes, which mimicked diabetic glomerulosclerosis and the reduced number of endothelial fenestrations. Transcriptional analyses and RT-PCR reported a twofold increase in Sema3a mRNA expression in Sema3g KO mice compared with wild-type glomeruli. Sema3a is involved in glomerular endothelial and podocyte development, and therefore, there may be some compensation mechanisms by other semaphorins in the absence of Sema3g.

**Conclusion:** We identified Sema3g as a novel podocyte-expressed gene that may be involved in the regulation of glomerular endothelial fenestrations and the structure of podocyte foot processes, together with other semaphorins.

## 1213

### Wound healing rate is not impaired in mice with deficiency of liver-derived insulin-like growth factor-I (IGF-I)

I.R. Botusan<sup>1,2</sup>, V.G. Sunkari<sup>1</sup>, J. Grunler<sup>1</sup>, J. Svensson<sup>3</sup>, J.-O. Jansson<sup>4</sup>, C. Ohlsson<sup>3</sup>, K. Brismar<sup>1</sup>, S.B. Catrina<sup>1</sup>;

<sup>1</sup>Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden, <sup>2</sup>Endocrinology Dept, Carol Davila UMPH, Bucharest, Romania, <sup>3</sup>Institute of Internal Medicine, Sahlgrenska Academy, Sweden, <sup>4</sup>Institute of Neuroscience and Physiology, Sahlgrenska Academy, Göteborg, Sweden.

**Background and aims:** IGF-I has growth promoting effects and it is expressed in virtually all tissues. Liver-derived IGF-I constitutes the major part of circulating IGF-I. Mice with deficiency of liver-derived IGF-I (LI-IGF-I<sup>-/-</sup> mice) have 75% lower serum levels of IGF-I, but approximately unaffected postnatal body growth. However, liver-derived IGF-I exerts several important effects in adult life such as regulation of cortical bone mass. IGF-I has a positive effect on wound healing and is decreased in wounds with depressed regenerative potential as diabetic wounds. However, the importance of circulating IGF-I on wound healing is unknown. Here, we investigate the role of systemic IGF-I on the wound healing rate in LI-IGF-I<sup>-/-</sup> mice during normoglycemic and diabetic conditions.

**Materials and methods:** LI-IGF-I<sup>-/-</sup> mice with complete inactivation of the IGF-I gene in the hepatocytes were generated by using the Cre/loxP recombination system. Mice homozygous for LoxP but lacking Mx-Cre were used as controls. Inactivation of the IGF-I gene in hepatocytes was performed at 1 month of age. Diabetes was induced with streptozotocin (50 mg/kg, i.p. five consecutive days) in LI-IGF-I<sup>-/-</sup> mice and in the controls. Wounds were made on the dorsum of animals, and evaluated by photos taken every second day. At the end of the experiment, wound biopsies were taken for being analyzed regarding granulation, dermal regeneration (hematoxylin eosin), angiogenesis (isolectin staining), markers for inflammation (CD45) and recruitment of endothelial precursor cells (EPC) (qRT-PCR). The IGF system (GH, IGF-II, IGF-R and IGFBP3) is analyzed in the skin biopsies at the mRNA (qRT-PCR) and/or protein level (immunohistochemistry, western blot).

**Results:** The wound healing rate was similar in the LI-IGF-I<sup>-/-</sup> mice compared with the controls (50% wound closure at  $4 \pm 0.5$  days versus  $4 \pm 0.3$  days,  $p = \text{ns}$ ). Diabetes significantly delayed the wound healing rate both in the LI-IGF-I<sup>-/-</sup> and control mice ( $p < 0.05$ ). However, no significant difference was observed between diabetic animals, with normal or reduced hepatic IGF production (50% wound closure  $6 \pm 0.2$  days - diabetic controls versus  $6 \pm 0.1$  days diabetic LI-IGF-I<sup>-/-</sup> mice).



**Conclusion:** Liver-derived IGF-I does not affect wound healing in mice, neither in normoglycemic conditions nor in diabetes.

*Supported by: Family Stefan Persson Foundation*

## 1214

### Notch signalling is activated in diabetes and contributes to defective wound healing

S.-B. Catrina<sup>1</sup>, V.G. Sunkari<sup>1</sup>, I.R. Botusan<sup>2</sup>, X. Zheng<sup>2</sup>, J. Grunler<sup>2</sup>, K. Brismar<sup>2</sup>;

<sup>1</sup>Department of Molecular Medicine and Surgery, <sup>2</sup>Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** Diabetic wounds are characterized by impaired coordination of several mechanisms essential for healing as cell proliferation, cell differentiation and angiogenesis. Notch system together with hypoxia is a major player in cell differentiation and angiogenesis. It consists of several receptors (Notch 1-4) and ligands with a high specific cell-dependent effect. Binding of the ligands to the receptors is followed by proteolytic cleavage of the receptor by  $\gamma$ -secretase complex which results in the release of intracellular domain of the Notch receptor (NICD) that translocates in the nuclei and activates several genes. We have proposed to study the modulation of the Notch pathway in diabetes and its potential relevance in defective diabetic wound healing.

**Methods:** The modulation of Notch system by hyperglycemia was studied *in vitro* in human dermal fibroblasts (HDF) and *in vivo* in several animal models (db/db mice and Goto-Kakizaki (GK) rat) using the corresponding technique (western blot, transitory transfections with reporter gene assay or evaluation of target genes by quantitative RT-PCR). The functional consequence of the notch system modulation was studied *in vitro* by assessment of the migration of HDF and by angiogenesis assay (in human endothelial cells). Notch pathway inhibition was induced either nonspecific by  $\gamma$ -secretase inhibitors (DAPT, L-685,458) or by specific siRNA silencing of the Notch receptors (1-4). The effect of the notch inhibition on wound healing in diabetes was evaluated in db/db mice by local treatment with  $\gamma$ -secretase inhibitors.

**Results:** Hyperglycemia activates Notch pathway at multiple levels. The repressive effect of high glucose on migration (fibroblasts) and angiogenesis (endothelial cells) was cancelled by blocking the notch signaling. Specific modulation of Notch receptors (1-4) using siRNA pointed out on a central role of Notch1 in mediating the repressive effect of hyperglycemia. Moreover local treatment with gamma secretase inhibitors improved the wound healing in db/db mice (percentage of wound closure on day 12 was 60 +/- 2% (DAPT), 70 +/- 4% (L-685,458) and 43 +/- 5 % (Placebo) respectively ( $p < 0.01$ ). Blocking Notch signaling in diabetic wounds was followed by increase in granulation and epidermal formation, increase in blood vessel formation and increase in expression of SDF-1 alpha responsible for recruitment of EPCs.

**Conclusion:** Hyperglycemia activated Notch system and significantly impaired cell migration and angiogenesis. Blocking notch system improved cell migration, angiogenesis and in consequence wound healing rate in diabetic animals.

*Supported by: Family Erling-Persson Foundation*

## 1215

### Skeletal muscle perfusion and mitochondrial oxidative activity in type 2 diabetic patients according to microangiopathic complications

S. Chiheb<sup>1</sup>, S. Duteil<sup>2</sup>, E. Cosson<sup>1</sup>, C. Wary<sup>2</sup>, J. Paries<sup>1</sup>, A. Monnet<sup>2</sup>, B. Chanu<sup>1</sup>, D. Mesangeau<sup>2</sup>, P. Carlier<sup>2</sup>, P. Valensi<sup>1</sup>;

<sup>1</sup>Department of Endocrinology-Diabetology-Nutrition, Jean Verdier Hospital AP-HP, CNRH-IdF, Paris-Nord University, Bondy, <sup>2</sup>Institut of Myology, NMR Laboratory, F-75651, CEA, I2BM, MIRCen, IdM NMR Laboratory, Paris, France.

**Background and aims:** We previously demonstrated that muscle perfusion and mitochondrial function were impaired in type 2 diabetic patients, especially in those with poor glycemic control and in those with multiple microangiopathic complications. The aim of this study was to assess the association of retinopathy, nephropathy and neuropathy with muscle perfusion and/or mitochondrial oxidative activity.

**Materials and methods:** We examined 96 type 2 diabetic patients, aged  $56.7 \pm 9.3$  years with body mass index  $27.5 \pm 4.1$  kg/m<sup>2</sup> and HbA1c  $7.6 \pm 1.8$  %, and without peripheral vascular disease. Multiparametric functional Nuclear

Magnetic Resonance (mpf-NMR) was performed to investigate muscle perfusion (Arterial Spin Labelled (ASL) NMR imaging (NMRI), oxygenation (dynamic proton NMR spectroscopy (<sup>1</sup>H-NMRS) of deoxymyoglobin (dMb) and mitochondrial function (spectroscopy <sup>31</sup>P) at rest, during and after an ischemic exercise of calf muscles. Symptoms and signs of peripheral neuropathy were graded using the Michigan Neuropathy Screening Instrument (MNSI). Vascular sympathetic activity was evaluated by the power of the low frequency spectrum of systolic blood pressure variations in the standing position (LF-SBP) using the Finapres<sup>®</sup> system.

**Results:** No relation was found between RMN parameters and retinopathy. Maximal muscle reperfusion after ischemic exercise correlated negatively with urinary albumin excretion rate ( $r = -0.328$ ,  $p = 0.002$ ) and was significantly lower in the patients with nephropathy ( $n = 24$ ) ( $p < 0.01$ ), even after adjustment on body mass index. Maximal muscle reperfusion was also lower in the patients with peripheral neuropathy (MNSI  $\geq 2.5/10$ ;  $n = 14$ ) ( $p = 0.057$ ). Re-oxygenation time of myoglobin correlated negatively with MNSI ( $r = -0.281$ ,  $p < 0.01$ ) and was longer (which means a defect of oxygenation) in the patients with peripheral neuropathy ( $p < 0.01$ ) than in those without. A defect of oxygenation was also associated with higher HbA1c levels ( $p < 0.01$ ) and lower vascular sympathetic activity ( $p = 0.01$ ), and remained associated with peripheral neuropathy after adjustment on age and HbA1c.

**Conclusion:** In type 2 diabetic patients the association between microalbuminuria and inadequate perfusion is consistent with the role of endothelial dysfunction in the impairment of muscle perfusion. The lower muscle oxygenation in the patients with peripheral neuropathy may result from a reduction in muscle sympathetic activity, possibly through a lower muscle perfusion.

*Clinical Trial Registration Number: NIH grant R21-DK063496*

*Supported by: NIH grant R21-DK063496*

## 1216

### Prevalence of liver histological abnormalities in type 1 diabetes and the long-term consequences

D.J. Harman<sup>1,2</sup>, R. Harris<sup>3</sup>, P. Kaye<sup>4</sup>, A. Gazis<sup>5</sup>, G.P. Aithal<sup>1,2</sup>;

<sup>1</sup>Hepatology, Nottingham University Hospitals NHS Trust, <sup>2</sup>NIHR NDDC Biomedical Research Unit, <sup>3</sup>General Medicine, Nottingham University Hospitals NHS Trust, <sup>4</sup>Histopathology, Nottingham University Hospitals NHS Trust, <sup>5</sup>Diabetes and Endocrinology, Nottingham University Hospitals NHS Trust, UK.

**Background and aims:** Patients with type 1 diabetes have a higher prevalence of raised liver enzymes than the general population. Ultrasound diagnosis of non-alcoholic fatty liver disease (NAFLD) has been reported to be common in type 1 diabetes despite associated insulin deficiency rather than insulin resistance. However, the histological spectrum of liver disease in type 1 diabetes and the natural history of chronic liver disease in this cohort is unknown. We describe the histological findings of patients with type 1 diabetes who had liver biopsy in a tertiary referral centre, and their long-term clinical outcome.

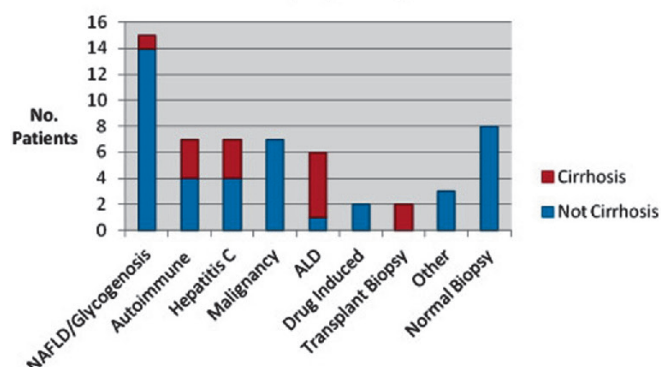
**Materials and methods:** The DIAMOND database, which contains longitudinal data for over 95% of patients with type 1 diabetes from an overall catchment population of 750,000 in Nottingham, England, was crossmatched with the clinical pathology database to identify those who had undergone liver biopsy. Case notes were reviewed to obtain follow up data, and identify liver and non-liver related outcomes.

**Results:** Out of 2,800 patients with type 1 diabetes, 57 patients underwent a total of 82 liver biopsies. Common indications for biopsy were abnormal liver enzymes (28 patients), malignancy (8), Hepatitis C staging (7) and clinical evidence of cirrhosis (3). On index biopsy, 86% had significant histological abnormalities (Figure 1) and 10 patients (17.5%) had cirrhosis. During a total follow up of 336.42 patient years (median 5.6 years), a further 4 patients developed cirrhosis, giving a cirrhosis prevalence of at least 500 per 100,000 population, compared with an estimated UK cirrhosis prevalence of 76.3 per 100,000 population. Portal hypertensive sequelae occurred in 11 patients (78.6%) with cirrhosis and hepatocellular carcinoma in 3 patients. Of those who had liver biopsy, 22 patients (38.6%) died during follow up. Non hepatic malignancy (9 patients), multiorgan failure (3) and liver failure (3) were the commonest mortality causes. Crude death rate was 6,539 per 100,000 person years, compared with national type 1 diabetes data of 1,878 per 100,000 person years. On age and sex adjusted multivariate analysis, diabetic nephropathy, raised GGT or prothrombin time, hypoalbuminaemia and non hepatic malignancy were associated with death.

**Conclusion:** Type 1 diabetes is associated with significant liver histology abnormalities and a higher than expected occurrence of cirrhosis, portal

hypertension and mortality. These findings have implications for long-term management of patients with type 1 diabetes.

### Liver Biopsy Diagnoses



## PS 107 Risk, pathophysiology and cardiovascular outcome in type 2 diabetes mellitus

1217

Comparison of Framingham risk score, UKPDS risk engine, maximum-IMT, and LDL-C/HDL-C ratio for predicting coronary plaque in asymptomatic patients with type 2 diabetes

K. Fujihara<sup>1</sup>, H. Suzuki<sup>2</sup>, A. Sato<sup>3</sup>, S. Kodama<sup>1</sup>, Y. Heianza<sup>1</sup>, T. Ishizu<sup>3</sup>, K. Saito<sup>1</sup>, H. Iwasaki<sup>1</sup>, K. Kobayashi<sup>2</sup>, S. Yato<sup>2</sup>, A. Takahashi<sup>2</sup>, N. Yamada<sup>4</sup>, H. Sone<sup>1</sup>, H. Shimano<sup>2</sup>;

<sup>1</sup>Endocrinology and Metabolism, University of Tsukuba, Mito,

<sup>2</sup>Endocrinology and Metabolism, University of Tsukuba, <sup>3</sup>Cardiology,

University of Tsukuba, <sup>4</sup>Internal Medicine, University of Tsukuba, Japan.

**Background and aims:** Although risk scores can predict future cardiovascular events, they fall short in predicting near-future events. Recent studies showed that the combination of the Framingham Risk Score (FRS) and carotid intima-media thickness (IMT) improved the prediction of cardiovascular disease.

**Materials and methods:** This study aimed to compare the predictability of FRS, UKPDS Risk Engine (UKPDS), maximum-IMT (max-IMT), and LDL-C/HDL-C ratio (L/H) for coronary plaque by 64-slice coronary CT angiography (CCTA) in asymptomatic patients with type 2 diabetes (T2DM) patients. A total of 116 Japanese T2DM patients who had a max-IMT  $\geq 1.1$  mm, electrocardiogram (ECG) abnormality, or positive exercise ECG test results underwent CCTA. Definitions of coronary artery stenosis and vulnerable coronary plaque were luminal narrowing of  $\geq 50\%$  and any coronary plaque with positive vessel remodeling and low attenuation, respectively. The 10-year risk of coronary heart disease was calculated according to the FRS and UKPDS. These 2 risk scores or clinical variables individually, a combination of either risk score and max-IMT or L/H, and the combination of max-IMT and L/H were compared to areas under the curve (AUCs) in receiver operating characteristic curve analysis. Trends of proportion of coronary artery stenosis through tertile of max-IMT in each category of the 2 risk scores were analyzed with generalized liner model.

**Results:** Of the 116 patients, 68 had coronary artery stenosis and 31 had vulnerable coronary plaque. The AUCs of each variables are listed in table. Figure shows a modification in the prediction of coronary artery stenosis based on max-IMT.

**Conclusion:** The data suggest that the combination of risk score with max-IMT improves the prediction of coronary plaque in comparison with a risk score alone and the combination of max-IMT and L/H is superior to a risk score alone in its simplicity in prediction of vulnerable coronary plaque.

AUCs for coronary artery stenosis, and vulnerable coronary plaque. Data are AUC (95% CI).

	Coronary artery stenosis	Vulnerable coronary plaque
FRS	0.763 (0.674-0.853)	0.655 (0.544-0.766)
FRS and Max-IMT	0.788 (0.705-0.872)	0.711 (0.607-0.815)
FRS and LDL/HDL	0.766 (0.676-0.856)	0.658 (0.541-0.776)
UKPDS	0.785 (0.703-0.868)	0.686 (0.579-0.794)
UKPDS and Max-IMT	0.800 (0.720-0.879)	0.719 (0.614-0.824)
UKPDS and LDL/HDL	0.785 (0.702-0.868)	0.690 (0.581-0.799)
Max-IMT and LDL/HDL	0.736 (0.644-0.827)	0.712 (0.605-0.819)

1218

Silent myocardial ischaemia and diabetes: to screen or not to screen!

A. Avignon<sup>1</sup>, J. Daures<sup>2</sup>, A. Sultan<sup>1</sup>;

<sup>1</sup>Nutrition-Diabetology, CHU Lapeyronie, <sup>2</sup>Biostatistic, IURC, Montpellier, France.

**Background and aims:** Given the elevated risk of cardiovascular events and the higher prevalence of silent myocardial ischemia (SMI) in diabetic versus non-diabetic patients, screening asymptomatic diabetic patients for CAD is an appealing concept. Indeed, a common argument for identifying SMI in

asymptomatic patients in general is to intensify treatment of risk factors and to propose a coronarography for eventual revascularization. However, published data demonstrating that a prospectively applied screening program improves outcome in asymptomatic diabetic patient are still lacking. Our objective was to determine if patients with SMI+ and coronarography have a better survival than the patients with SMI who did not undergo coronarography. Survival was compared to the patients without SMI.

**Materials and methods:** In our department, we used to screen for silent myocardial ischemia in high risk diabetic patients, following the international recommendations. If SMI was diagnosed, a coronary angiography was indicated. All patients benefits of treatment of their risk factors following international guidelines.

**Results:** Since 2000, 913 high risk diabetic patients (706 type 2 diabetic patients and 207 type 1 diabetic patients) with normal baseline electrocardiography (ECG) were evaluated to identify patients with SMI, using myocardial perfusion imaging. 171 patients had SMI (SMI prevalence 18%). Coronarography was done in 94 patients SMI+. There was no difference regarding risk factors, nor diabetic complications nor treatment between the group who underwent coronarography and the one who did not. These patients were followed during a mean period of 6,5 years. Survival analysis was made for univariate analysis, using Kaplan Meyer methods. Covariables with  $p$ -value  $< 0.15$  at log-rank test were introduced in COX multivariate analysis. Significant variables were selected for final model selection through STEPWISE, BACKWARD, FORWARD methods. 79 patients died (8,6%), mainly from cardiovascular diseases (84%). In univariate analysis, presence of SMI was not associated with total mortality ( $p=0,2263$ ) nor cardiovascular mortality ( $p=0,57$ ). Furthermore, in the group of patients SMI+, coronarography was not associated with a better survival in multivariate analysis.

**Conclusion:** In conclusion, this observational study should argue for intensive cardiovascular risk factors treatment in diabetes in reducing cardiovascular mortality, with no argument for a benefit from SMI screening.

## 1219

### Activin A is derived from human epicardial adipose tissue and associates with decreased myocardial function and glucose metabolism in human diabetic cardiomyopathy

W.J.Y. Chen<sup>1</sup>, S. Greulich<sup>2</sup>, L.J. Rijzewijk<sup>1</sup>, R.W. van der Meer<sup>3</sup>, M. Bekaert<sup>4</sup>, H.J. Lamb<sup>3</sup>, A. de Roos<sup>3</sup>, J.A. Romijn<sup>5</sup>, J.W.A. Smit<sup>3</sup>, G. Vandenplas<sup>4</sup>, A.A. Lammertsma<sup>1</sup>, M. Lubberink<sup>1</sup>, J.B. Ruige<sup>4</sup>, M. Diamant<sup>1</sup>, D.M. Ouwens<sup>2</sup>; <sup>1</sup>VU University Medical Center, Amsterdam, Netherlands, <sup>2</sup>German Diabetes Center, Duesseldorf, Germany, <sup>3</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>4</sup>University Hospital Ghent, Belgium, <sup>5</sup>Academical Medical Center, Amsterdam, Netherlands.

**Background and aims:** Previously, we found that an enhanced release of activin A from epicardial adipose tissue in patients with type 2 diabetes (T2D) impairs cardiomyocyte function *in vitro*. The aims of the present study were to (1) determine activin A levels in intracoronary sinus and peripheral serum samples of both patients with and without T2D undergoing coronary artery bypass grafting (CABG), and (2) assess the association of systemic activin A levels with cardiovascular and metabolic parameters in both asymptomatic men with uncomplicated T2D and healthy controls.

**Materials and methods:** Serum activin A levels were determined in the two distinct cohorts by ELISA. Cohort 1 included 18 T2D patients and 9 age- and sex-matched non-T2D patients undergoing CABG. Cohort 2 involved 78 T2D men in whom inducible ischaemia had been excluded and 16 age-matched healthy men. Cardiac function was measured using magnetic resonance imaging, and myocardial glucose metabolism (MMRglu) was determined using <sup>18</sup>F-2-fluoro-2-deoxy-D-glucose positron emission tomography during a hyperinsulinaemic-euglycaemic clamp.

**Results:** In patients undergoing CABG, activin A levels were higher in the intracoronary sinus than in peripheral blood ( $1861 \pm 397$  vs  $270 \pm 84$  pg/mL,  $P < 0.001$ ). T2D patients had higher activin A levels in the intracoronary sinus than the non-T2D patients ( $1955 \pm 415$  vs  $1670 \pm 206$  pg/mL,  $P < 0.05$ ), whereas peripheral activin A levels were similar between both groups. In cohort 2, compared with controls, T2D patients had higher body mass index (BMI), systolic blood pressure (SBP), pulse wave velocity (PWV), left ventricular (LV) mass/volume ratio, and decreased LV diastolic function, clamp-derived insulin sensitivity and MMRglu (all  $P < 0.05$ ). Peripheral activin A levels were similar to cohort 1, and did not differ between T2D patients and controls. Yet, a significant negative association was found between activin A levels and MMRglu ( $r = -0.450$ ,  $P < 0.001$ ), but not whole-body insulin sensitivity in T2D patients. In a multivariate analysis, adjustment for age, BMI, insulin sensitiv-

ity, fasting glucose and insulin levels, did not affect this association. Furthermore, there was a positive association of activin A with LV mass/volume ratio and PWV. Whilst age and SBP were confounding factors for the relation with PWV ( $P = 0.13$ ), the association between activin A and LV mass/volume ratio remained significant after adjustment for age, SBP and BMI ( $P = 0.02$ ).

**Conclusion:** Intracoronary sinus, but not peripheral activin A levels are elevated in T2D patients vs non-T2D patients undergoing CABG. Yet, in T2D, peripheral activin A levels negatively associate with MMRglu and positively with LV mass/volume ratio. These data suggest that activin A may not only represent a useful biomarker, but may also be a causal factor in the development of diabetic cardiomyopathy.

Clinical Trial Registration Number: ISRCTN53177482

Supported by: Eli Lilly, the Netherlands

## 1220

### Adenosine triphosphate binding cassette transporter G1 expression in monocytes is associated with HDL-carboxymethyllysine in type 2 diabetes

K.C.B. Tan, J.G.S. Tsun, S.W.M. Shiu, Y. Wang, Y. Wong, S.S.Y. Yung, D.T.M. Chan;

Department of Medicine, The University of Hong Kong

**Background and aims:** Dyslipidemia in type 2 diabetes is characterized by hypertriglyceridemia and low HDL cholesterol level. In addition to quantitative changes in HDL, qualitative abnormalities in HDL particles have also been reported. Glycation of HDL is increased and may affect the anti-atherogenic property of HDL. For instance, advanced glycation of apo A1 impairs its ability to promote cholesterol efflux and to stabilize adenosine triphosphate binding cassette transporter A1 (ABCA1) *in vitro*. We aimed to evaluate the extent of advanced glycation in HDL by measuring N(epsilon)-(carboxymethyl)lysine (CML) in HDL and determine the impact on the anti-oxidative property of HDL and cholesterol transporters expression in monocytes.

**Materials and methods:** 30 type 2 diabetic patients and 30 matched controls were recruited. CML content in HDL was measured by sandwich ELISA. mRNA of cholesterol transporters ABCA1, ABCG1, and scavenger receptor class B type I (SR-BI) in peripheral monocytes was determined by real time quantitative PCR. The capacity of HDL to inhibit LDL oxidation *ex vivo* was determined by incubating HDL with a reference LDL in the presence of dichlorofluorescein which fluoresced upon interaction with lipid oxidation products.

**Results:** Diabetic patients had higher levels of HDL-CML than controls [ $5626$  ( $2493$ – $11826$ ) ng/mg vs  $4453$  ( $806$ – $7263$ ), median (interquartile range),  $p < 0.01$ ]. The ability of HDL from diabetic patients in protecting LDL against oxidation was impaired with more lipid peroxide formed during the incubation ( $3260 \pm 669$  fluorescence intensity vs  $2840 \pm 642$ ,  $p < 0.05$ ). The expression of ABCG1 in monocyte was decreased in diabetic patients ( $p < 0.05$ ), whereas the levels of ABCA1 and SR-BI were comparable between the two groups. There was an inverse correlation between serum levels of HDL-CML and monocyte ABCG1 expression ( $r = -0.32$ ,  $p = 0.01$ ) but no correlation was seen with ABCA1, SR-BI expression or with HDL anti-oxidative capacity.

**Conclusion:** Diabetic patients have increased amount of CML in HDL and the antioxidant property of HDL is impaired. There is an association between HDL-CML and the expression of ABCG1 in peripheral monocytes. Whether the expression of ABCG1 is directly influenced by HDL-CML warrants further investigation.

Supported by: RGC grant HKU776709M

## 1221

### Diabetics show better collaterals toward the culprit vessel at the time of their first acute coronary event

D. D'Amario, G. Zaccardi, G. Niccoli, S. Giubilato, A. Leo, N. Cosentino, D. Pitocco, G. Ghirlanda, F. Crea; Catholic University, Rome, Italy.

**Background and aims:** Diabetes is associated with a more extensive and severe coronary artery disease (CAD), however similar age of presentation of non diabetics has been described despite a more extensive CAD at the time of a first acute coronary syndrome (ACS), thus suggesting that protective mechanisms may act in diabetics.

**Materials and methods:** We evaluated angiographic CAD severity and quality of the collateral circulation toward the culprit vessel according to the



diabetic status in consecutive patients at their first ACS. One hundred and sixty-seven consecutive patients undergoing coronary angiography because of non-ST-elevation (NSTEMI)-ACS (53% unstable angina and 47% NSTEMI-myocardial infarction), as their first clinical manifestation of CAD, and showing obstructive atherosclerosis were prospectively included. CAD severity was graded according to Bogaty's stenosis score and extent index, while collateral filling was graded according to Rentrop classification toward culprit vessel showing a TIMI flow  $\leq 2$ .

**Results:** Forty-seven patients (28%) were diabetics. Diabetics and non diabetics were similar for age ( $69 \pm 12$  vs  $66 \pm 14$ ,  $p=0.11$ ). Multivessel disease rate was higher in diabetics as compared to non diabetics (68% vs 42%,  $p=0.003$ ). At multivariate analysis both stenosis score and extent index were predicted by diabetes ( $p=0.001$ ), stenosis score was predicted by male sex ( $p=0.03$ ) and extent index by lack of statins ( $p=0.05$ ). Good collaterals (Rentrop 2-3) were observed in 28 over 83 culprit vessel with a TIMI flow  $\leq 2$ . At multivariate analysis, good collaterals were predicted by diabetes (OR 2.33, 95% CI 1.45–6.54,  $p=0.002$ ) and inversely by extent index (OR 0.87, 95% CI 0.51–0.96,  $p=0.03$ ).

**Conclusion:** Diabetics show at their very first ACS a more extensive and severe coronary atherosclerosis as compared to non diabetics, despite a similar age of presentation. However, angiographically visible collaterals toward the culprit vessel are better developed in diabetics, suggesting that collaterals may delay onset of a first ACS acting as a protective mechanisms.

## 1222

### Hyperuricaemia as an independent predictor of vascular complications and mortality in diabetic patients: a meta-analysis

Y. Xu<sup>1</sup>, J. Bai<sup>2</sup>, Y. Liu<sup>3</sup>, X. Wu<sup>1</sup>, C. Shen<sup>2</sup>;

<sup>1</sup>Endocrinology, the First Affiliated Hospital with Nanjing Medical

University, <sup>2</sup>Epidemiology & Statistics, Nanjing Medical University,

<sup>3</sup>Geriatrics, the First Affiliated Hospital with Nanjing Medical University, China.

**Aims:** Diabetes Mellitus (DM) is undoubtedly one of the most challenging health problems in the 21st century and the number of diabetic patients diagnosed has reached 366 million in 2011. Complications due to diabetes are a major cause of disability, reduced quality of life and death. Much epidemiologic evidence was committed to risk factors related to development of DM and its complications. Elevated serum uric acid (SUA) or hyperuricemia (HUA), a member of metabolic syndrome, has been reported to be an independent risk factor for the development of DM. Recent series reports indicated different role of HUA in complications attributed to DM. Hence, a pooled analysis of those results of the relationship between elevated SUA and diabetic complications would be warranted. Our aim is to ascertain the role of elevated SUA in the development of complications in DM.

**Materials and methods:** A computerized literature search of Medline database, Embase database, and Pubmed database was conducted and the odds ratio (OR) and hazard ratio (HR) for per 1mg/dl increase in SUA in each study was calculated for some studies defined SUA as a categorical variable. Cochran's Q and  $I^2$  statistics were used to evaluate heterogeneity among studies and pooling odds ratio (OR) and hazard ratio (HR) with 95% confidence intervals (CIs) were calculated using random-effects models and fix-effects models. The pooled analysis was performed with STATA 10.0.

**Results:** In total, 11 articles (19 ORs and RRs) including 22,591 participants were included in the meta-analysis. In the forest plot, pooled estimates for the relationship suggested that each 1 mg/dl increase in SUA resulted in a 17.4% increase in the risk of diabetic vascular complications and a 10.8% increase in the risk of diabetic mortality. In the stratified-analysis, HUA was markedly associated with diabetic macro- and microvascular complications, the pooled ORs (95% CI) were 1.004 (1.001–1.008) and 1.684 (1.289–2.200), respectively. HUA was more common in patients with nephropathy, the risk of which would be double (2.019 [1.268–3.449]) with each 1 mg/dl increase in SUA. Moreover, the overall RR and HR were inconsistently significant across different populations and sample size. The positive correlation between HUA and vascular complications remained in Asian (1.333 [1.005–1.770]) and European population (1.765 [1.284–2.426]), whereas, not in Australian population (1.252 [0.820–1.911]). And this also happens in mortality attributed to HUA in European (HR [95% CI] equaled to 1.265 [1.105–1.448]) and Australian population (1.054 [0.969–1.146]). Studies with relative large sample size over 1000 presented a significant association between HUA and diabetic vascular complications and HR (95% CI) was 1.410 (1.074–1.852), while those with small sample size (< 1000) failed in positive association, HR (95% CI) was 1.410 (1.074–1.852).

**Conclusion:** Results of this meta-analysis supported that hyperuricemia was an independent predictor of vascular complications and mortality in diabetic patients, thus lowering SUA levels should be helpful for prevention and treatment of complications in DM.

Supported by: NSFC81070621

## 1223

### Reduction in stroke during intensified multifactorial treatment in microalbuminuric type 2 diabetic patients

J. Ølgaard<sup>1,2</sup>, F. Persson<sup>3</sup>, P. Rossing<sup>3</sup>, H.-H. Parving<sup>4,5</sup>, O. Pedersen<sup>6,7</sup>, P. Gæde<sup>2</sup>;

<sup>1</sup>Faculty of Health Science, University of Copenhagen, <sup>2</sup>Dept. of Internal

Medicine, Slagelse University Hospital, Slagelse, <sup>3</sup>Steno Diabetes Center,

Gentofte, <sup>4</sup>Dept. of Medical Endocrinology, Rigshospitalet, Copenhagen,

<sup>5</sup>Faculty of Health Science, Aarhus University, <sup>6</sup>Novo Nordisk Foundation

Center for Basic Metabolic Research, Faculty of Health Sciences,

Copenhagen, <sup>7</sup>Hagedorn Research Institute, Gentofte, Denmark.

**Background and aims:** Patients with type 2 diabetes have a high risk for stroke compared to non-diabetic patients and have a significantly poorer prognosis for long-term survival and disability leading to major personal and economic burden. Several risk factors have been identified, and in the Steno 2 study the intensive, multifactorial intervention approach has earlier been demonstrated to significantly lower the cardiovascular risk. This study aimed to compare the risk for stroke in the conventional and the intensively treated cohort in the Steno 2 Study.

**Materials and methods:** 160 type 2 diabetic patients with microalbuminuria (u-albumin 30–300mg/l) were randomized to conventional (n = 80) or multifactorial, intensified treatment. Patients in the conventional arm were followed according to national guidelines for treatment of diabetes. Patients in the intensive arm, treatment targeted several concomitant risk factors with both pharmacological and behavioral intervention and a special task group followed the patients at our center. Stroke was defined according to prespecified criteria and adjudicated by an independent committee whose members were unaware of the patients' treatment assignments.

**Results:** Mean follow up was 7.8 yrs. During follow up three patients in the intensive group experienced a total of three strokes (recurrence rate = 0%) and 11 patients in the conventional arm experienced 20 strokes (mean = 1.8, recurrence rate = 55%). Intensive intervention reduced the relative risk for stroke by 75% (unadjusted relative risk was 0.25 (0.07–0.89)) and absolute risk of stroke by 10%. Adjustment for risk factors did not alter this value significantly. Allocation to intensive treatment and baseline systolic blood pressure was identified as significant independent risk factors for stroke in a Cox regression analysis: HR 0.23 (0.07–0.84,  $p=0.026$ ) and HR 1.03 (1.01–1.06,  $p=0.009$ ), respectively. Baseline fasting HDL-cholesterol was of borderline significance (HR 0.10 (0.01–1.53,  $p=0.09$ ). There was no significant impact of baseline glycaemic control, UAER, eGFR, smoking, age and gender or diabetes duration.

**Conclusion:** Intensified multifactorial intervention is associated with significantly reduced risk for first and recurrent stroke in the Steno 2 cohort of microalbuminuric type 2 diabetic patients. This finding further emphasizes the importance of multifactorial intervention for significant and cost-effective cardiovascular protection.

## 1224

### Significant improvement of VO2 peak, after myocardial infarction, in type 2 diabetes when glycaemic level is well controlled during cardiac rehabilitation: the DARE Study

B. Vergès<sup>1</sup>, B. Patois-Vergès<sup>2</sup>, I. Robin<sup>1</sup>, J. Bertrand<sup>3</sup>, J. Feige<sup>3</sup>, M.-C. Iliou<sup>4</sup>, H. Douard<sup>5</sup>, B. Catargi<sup>5</sup>, M. Fishbach<sup>6</sup>, B. Delenne<sup>7</sup>, C. Cabanot-sarrau<sup>7</sup>, B. Pierre<sup>8</sup>;

<sup>1</sup>Hôpital du Bocage, Dijon, <sup>2</sup>Les Rosiers, Dijon, <sup>3</sup>Polyclinique Urban V,

Avignon, <sup>4</sup>Hôpital Broussais, Paris, <sup>5</sup>CHU Bordeaux, Bordeaux, France,

<sup>6</sup>Polyclinique Bordeaux Nord, Dijon, <sup>7</sup>CH Aix en Provence, <sup>8</sup>Centre Iris,

Marcy-l'Étoile, France.

**Background and aims:** It is well known that gain in VO2 peak, after cardiac rehabilitation (CR), is associated with reduced mortality and morbidity. Our group has previously shown that the VO2 peak improvement in CR, after an acute ischaemic heart event, is significantly lower in Type 2 diabetic patients and that response to CR seems to be impaired by poor glycaemic control.

**Materials and methods:** Thus, we set up a prospective multicenter study (DARE Study) in order to determine whether good glycemic control during CR may improve the gain in VO<sub>2</sub> peak. Fifty six patients with type 2 diabetes, HbA<sub>1c</sub>>7% and referred to CR after a myocardial infarction occurring during the previous month were randomized in an intensive treatment group with basal-bolus insulin therapy or in a control group in which the antidiabetic treatment at time of enrolment in the study was maintained. Final fructosamine, at the end of the CR program, was used to assess mean glycemic level during the 3–4 week CR period.

**Results:** For the whole diabetic population studied, the mean gain in VO<sub>2</sub> peak after CR was  $2.7 \pm 2.4$  ml/kg/min in absolute value and  $16 \pm 15$  % in relative value. Gain in VO<sub>2</sub> Peak after CR was negatively correlated with basal ( $r=-0.31$ ,  $p<0.05$ ) and final fructosamine ( $r=-0.36$ ,  $p=0.01$ ) and with basal ( $r=-0.35$ ,  $p<0.05$ ) and final HbA<sub>1c</sub> ( $r=-0.35$ ,  $p<0.05$ ). At the end of the CR program, the gain in VO<sub>2</sub> peak and the reduction in mean fructosamine level were not significantly different between the 2 treatment groups. But, patients who had final fructosamine level below the median value showed significantly higher gain in VO<sub>2</sub> peak in absolute value ( $3.5 \pm 2.5$  vs.  $1.7 \pm 2.4$  ml/kg/min,  $p=0.014$ ) and relative value ( $+20 \pm 13$  vs.  $11 \pm 14$  %,  $p=0.048$ ). In multivariate analysis gain in VO<sub>2</sub> peak was associated with final fructosamine level ( $p=0.028$ ) but not with age, gender, duration of diabetes, MI location, insulin treatment or basal fructosamine.

**Conclusion:** The DARE study shows that fructosamine level at the end of the CR-program is an important determinant of gain in VO<sub>2</sub> peak in patients with type 2 diabetes and that good glycemic control in CR is associated with significantly better gain in VO<sub>2</sub> peak, independent of the treatment used (insulin or not). These data indicate that good glycemic control of type 2 diabetes in CR, after myocardial infarction, is mandatory in order to get optimal gain in VO<sub>2</sub> peak.

Clinical Trial Registration Number: NCT00354237

Supported by: ALFEDIAM and NovoNordisk

## 1225

### Glycosylated haemoglobin influences myocardial perfusion after pharmaco-invasive therapy

L.N. Matos, D. Rangel, F. Homem, J. Diniz, F. Perez, J.L. Herrmann, J.A. Marconi, I. Goncalves Jr, E. Stefanini, A.C. Carvalho;  
Cardiology Department, Federal University of Sao Paulo, Brazil.

**Background and aims:** Diabetes mellitus is a condition that is associated with coronary microvascular disease. The role of glycosylated hemoglobin (HbA<sub>1c</sub>) levels determined on admission of patients with acute ST elevation myocardial infarction (STEMI) remains unclear. We aimed to determine the metabolic variables, including serum levels of HbA<sub>1c</sub>, possibly correlated with coronary microvascular permeability observed after treatment with pharmaco-invasive (PI) strategy in patients who had STEMI.

**Materials and methods:** We evaluated consecutive patients with STEMI referred from low complexity health care units to tertiary care general hospital after thrombolysis with teneceplase (TNK) for performing coronary angiography within 6–24 hours (PI strategy). All subjects received TNK up to six hours after onset of STEMI symptoms. Were determined, after eight hours fasting, serum glucose, HbA<sub>1c</sub>, TSH, total cholesterol, HDL-cholesterol, and triglycerides, LDL-cholesterol was determined through Friedewald formula. The myocardial blush grade (MBG) was determined (Gibson technique) by a single operator, experienced, at the beginning of the coronary angiography, and after PI therapy. Myocardial perfusion was regarded as either present (MBG 2 or 3) or absent (MBG zero or 1). We selected individuals who had no myocardial perfusion at the beginning of the coronary angiography, which were divided into groups as follows: individuals who still had no myocardial perfusion (group 1) and individuals who had gained adequate myocardial perfusion (group 2) after cath.

**Results:** We evaluated 113 individuals, of whom 51 (45.1%) had no myocardial perfusion initially, with thirty in group 1 (24 men,  $58.6 \pm 11.2$  years), and 21 in group 2 (16 men,  $54.0 \pm 11.7$  years). Group 1 subjects, compared to group 2, had higher HbA<sub>1c</sub> levels ( $7.49 \pm 2.15$  vs.  $6.12 \pm 1.01$ ,  $p = 0.02$ ). There was no significant difference between the two groups for the other parameters analyzed.

**Conclusion:** We observed that individuals without myocardial perfusion after FI strategy showed higher HbA<sub>1c</sub> levels at admission. This parameter, known to be associated with coronary microvascular disease, may be related to the failure of the restoration of flow in the coronary microcirculation in the sample studied.

## 1226

### Cardiac response to 2 years of intensified structured multi-intervention vs standard care in type 2 diabetes: a randomised trial

A.P. Ofstad<sup>1</sup>, O.E. Johansen<sup>1</sup>, K.I. Birkeland<sup>2</sup>, L. Gullestad<sup>3</sup>, S. Urheim<sup>3</sup>, S. Aakhus<sup>3</sup>;

<sup>1</sup>Medical Research Department, Asker and Baerum Hospital, Vestre Viken Hospital Trust, Baerum, <sup>2</sup>Department of Endocrinology, Oslo University Hospital Aker, <sup>3</sup>Department of Cardiology, Oslo University Hospital Rikshospitalet, Norway.

**Background and aims:** Patients with type 2 diabetes (T2D) are at elevated risk for heart failure (HF) and premature cardiovascular (CV) mortality. However, despite a strong epidemiological link, there is data suggesting that intensive glucose lowering could in fact be associated with worsened HF and CV outcomes. Since this remains a controversial topic, we hypothesized that an intensified structured multiintervention program (STRUCT) aimed against multiple HF and CV risk factors (hyperglycaemia, dyslipidaemia and hypertension) would improve, or at least not be harmful to, myocardial function compared to conventional standard care (STAND).

**Materials and methods:** 100 patients with T2D and  $\geq 1$  CV risk factor (29% females, mean age (mean $\pm$ SD)  $58 \pm 10$ , diabetes duration  $6 \pm 6$  yrs, BMI  $30.1 \pm 5.5$  kg/m<sup>2</sup>, 12% coronary artery disease, 53% self-reported dyspnoea, HbA<sub>1c</sub>  $7.6 \pm 1.6$ %, LDL  $2.9 \pm 0.9$  mmol/L, blood pressure (BP)  $141 \pm 18/83 \pm 9$  mmHg, were randomized to STRUCT (n=50) or STAND (n=50) care for 2 years. The groups were well balanced at baseline (BL) in all measured variables except prevalence of microalbuminuria (STRUCT 62%/STAND 22%). STRUCT comprised lifestyle intervention and targeted pharmacological treatment to reach pre-specified targets (HbA<sub>1c</sub>  $\leq 6.5$ %, total/LDL cholesterol  $<5/3$  mmol/L, BP  $<130/80$  mmHg) whereas the STAND group remained under GP care following current guidelines. All underwent echocardiography (echo) including Tissue Doppler Imaging (TDI) at BL. 90 completed the study with repeated echo. Blood samples were drawn and cardiopulmonary exercise test (CPET) performed at BL and 2 years.

**Results:** The STRUCT group obtained a significant improvement in lipids and glycaemic control as compared to the STAND group (reductions at study end for HbA<sub>1c</sub>:  $-0.8 \pm 1.4/+0.2 \pm 1.4$  % [ $p=0.001$ ], LDL:  $-0.7 \pm 0.9/-0.4 \pm 0.7$  mmol/L [ $p=0.13$ ], triglycerides:  $-0.5 \pm 1.3/0.1 \pm 0.7$  mmol/L [ $p=0.012$ ], respectively), whereas reductions in BP were similar. At BL, E/e', a measure of left ventricular (LV) filling pressure, was elevated ( $13.3 \pm 4.7$ ; reference value:  $<8$ ). Parameters of systolic function was preserved over 2 years in both groups (Table). Whereas, E deceleration time increased in the STAND group, and E/A ratio increased in the STRUCT group, there were no significant differences in the overall group responses in diastolic function. Peak work capacity defined as max watt obtained on CPET, was improved in STRUCT group and decreased in STAND group (between-group  $\Delta$ :  $9.2 \pm 3.4$  W,  $p=0.007$ ).

**Conclusion:** Two years of intensified STRUCT intervention in patients with T2D improved HF risk factors and work capacity, and did not worsen systolic or diastolic LV function.

Echocardiographic findings. Mean $\pm$ SD, p-value indicate between-group difference in  $\Delta$ .

	STRUCT group			STAND group			p-value ( $\Delta$ STRUCT vs $\Delta$ STAND)
	BL (n=50)	2 years (n=44)	$\Delta$	BL (n=50)	2 years (n=46)	$\Delta$	
Systolic function							
EF (%)	62 $\pm$ 8	63 $\pm$ 6	0.5 $\pm$ 8.7	63 $\pm$ 7	62 $\pm$ 8	-1.7 $\pm$ 7.6	0.21
Peak systolic velocity (s') cm/s	5.5 $\pm$ 1.5	5.6 $\pm$ 1.2	0.2 $\pm$ 1.4	5.5 $\pm$ 1.5	5.4 $\pm$ 1.4	-0.1 $\pm$ 1.5	0.32
Peak systolic longitudinal displacement (mm)	10.1 $\pm$ 2.2	10.5 $\pm$ 2.1	0.4 $\pm$ 2.3	10.0 $\pm$ 2.2	10.0 $\pm$ 2.1	-0.2 $\pm$ 1.6	0.14
Diastolic function							
Deceleration time (ms)	195 $\pm$ 57	211 $\pm$ 49	11 $\pm$ 58	194 $\pm$ 42	215 $\pm$ 43	22 $\pm$ 48**	0.32
E/A-ratio	0.89 $\pm$ 0.28	1.01 $\pm$ 0.36	0.13 $\pm$ 0.29**	0.93 $\pm$ 0.27	0.95 $\pm$ 0.25	0.03 $\pm$ 0.21	0.067
Septal early diastolic velocity (e') (cm/s)	5.0 $\pm$ 1.7	5.2 $\pm$ 1.8	0.2 $\pm$ 1.8	5.4 $\pm$ 1.8	5.0 $\pm$ 1.8	-0.4 $\pm$ 1.7	0.10
E/e' septal	13.5 $\pm$ 5.0	14.1 $\pm$ 5.8	1.0 $\pm$ 5.5	13.1 $\pm$ 4.6	13.8 $\pm$ 4.2	0.9 $\pm$ 4.3	0.98

\*, \*\*, \*\*\*:  $p < 0.05, 0.01, 0.001$  vs baseline. Values are mean $\pm$ SD.

Clinical Trial Registration Number: NCT00133718

Supported by: Norwegian Health Authority Southeast

## PS 108 Pharmacological aspects and cardiovascular outcome

1227

### Comparison of clinical and economic outcomes associated with DPP4i versus SU in combination with MET or PIO for the treatment of type 2 diabetes mellitus

K. Chen<sup>1</sup>, E. Wu<sup>1</sup>, D. Cheng<sup>1</sup>, A. Bensimon<sup>1</sup>, M. Bron<sup>2</sup>

<sup>1</sup>Analysis Group, Inc., Boston, <sup>2</sup>Global Health Economics and Outcomes Research, Takeda Pharmaceuticals International, Inc., Deerfield, USA.

**Background and aims:** To compare diabetes-related complications and healthcare utilizations and costs between diabetes patients treated with DPP4 inhibitors (DPP4i)-based combinations [DPP4i+metformin (MET) or DPP4i+pioglitazone (PIO)] vs. sulfonylurea (SU)-based combinations (SU+MET or SU+PIO).

**Materials and methods:** Data from MarketScan, a large U.S. commercial claims database, were analyzed to retrospectively compare outcomes between patients treated with DPP4i and SU combinations. The study sample consisted of patients with type 2 diabetes mellitus (T2DM) who were treated with DPP4i or SU combinations and had continuous eligibility from 6 months prior to the index date (date of combination therapy initiation) to 12 months following the index date. Rates of diabetic complications, healthcare utilization, and costs in the 12 month period following index date were compared between the treatment groups. Complications of interest included microvascular complications (e.g. diabetic retinopathy), macrovascular complications (e.g. atherosclerosis), and other diabetic complications. Cox proportional hazards models were used to compare the rates of complications and adjust for baseline characteristics. Generalized linear models or two-part models (in outcomes with excessive 0's) were similarly used for diabetes-related utilizations and costs.

**Results:** 56,741 patients were selected into the study sample (n=15,139 receiving DPP4i combinations; n=41,602 receiving SU combinations). At baseline, patients treated with DPP4i combinations exhibited higher rates of comorbidities. After adjustment for baseline characteristics including demographics, diabetes-related complications, comorbidities, utilization and costs, the hazard ratios of complications were significantly lower for patients treated with DPP4i combinations (microvascular complications: 0.872,  $P<0.0001$ ; macrovascular conditions: 0.933,  $P=0.0015$ ; other complications: 0.921,  $P<0.0001$ ). DPP4i patients also had lower adjusted diabetes-related utilization incidence rate ratios (hospitalization: 0.76; ER visits: 0.69; outpatient visits: 0.87; all  $P<0.0001$ ). DPP4i patients incurred higher mean diabetes-related prescription drug costs (\$1,629 vs. \$476,  $P<0.0001$ ) but lower all-cause medical services costs (\$6,731 vs. \$7,251,  $P<0.0001$ ).

**Conclusion:** While patients on DPP4i combinations incur higher diabetes-related drug costs, they were found to have lower all-cause medical services costs than patients treated with SU combinations. However, the cost-savings from medical services associated with DPP4i combination therapy were less than the incremental drug cost. Lastly, our study found that DPP4i-combination patients had lower rates of diabetes-related complications and healthcare utilization in the 12 month follow up period, compared to those treated with SU combinations.

Supported by: Takeda Pharmaceuticals, Inc.

1228

### Pioglitazone and macrovascular outcomes during observational follow-up of PROactive: 6-year update

E. Erdmann<sup>1</sup>, E. Song<sup>2</sup>, R. Spanheimer<sup>3</sup>, A.-R. Van Troostenburg de Bruyn<sup>2</sup>, A. Perez<sup>2</sup>

<sup>1</sup>Medical Clinic III, University of Cologne, Germany, <sup>2</sup>Takeda Global Research & Development Center, <sup>3</sup>Takeda Pharmaceuticals North America, Deerfield, USA.

**Background and aims:** In PROactive, a double-blind, placebo-controlled outcomes study in type 2 diabetes mellitus (T2DM) patients with known macrovascular disease, pioglitazone provided a non-statistically significant 10% relative risk reduction for the primary composite endpoint (all-cause death, myocardial infarction [MI], acute coronary syndrome [ACS], cardiac intervention, stroke, major leg amputation, leg revascularization) and a statistically significant 16% reduction for the main secondary endpoint (death, MI, stroke) after a mean 34.5 mo. Of 5238 randomized patients, 3599 (74%)

subsequently entered a 10-yr non-interventional observational study with no exclusion criteria or allocation to study medication; patients are treated per standard medical practice.

**Materials and methods:** An interim 6-yr analysis used a Cox proportional hazard model to compare non-adjudicated macrovascular events (using the same endpoints excluding ACS) between pioglitazone and placebo based on patients' original randomization in PROactive.

**Results:** For the observational period alone (mean 5.8 yr, during which fewer than 15% of patients received pioglitazone at any time) or the combined PROactive double-blind and 6-yr observational period (up to 9.5 yr in total; mean 8.7 yr), there were no statistically significant differences in the primary or secondary endpoints for pioglitazone *versus* placebo (Table).

**Conclusion:** These results suggest that the improved cardiovascular outcome seen with pioglitazone during the double-blind period of PROactive did not persist during the observational period in the absence of continued pioglitazone treatment.

Treatment Period	Endpoint	Original treatment during double-blind period		Hazard ratio	95% CI	p-value
		Pioglitazone	Placebo			
Double-blind (PROactive, mean 34.5 mo)	Primary	(n=2605) 514 (19.7%)	(n=2633) 572 (21.7%)	0.90	[0.80, 1.02]	0.095
	Main secondary	301 (11.6%)	358 (13.6%)	0.84	[0.72, 0.98]	0.027
	All-cause death	177 (6.8%)	186 (7.1%)	0.96	[0.78, 1.18]	0.678
Observational only (mean 5.8 yr)	Primary	(n=1820) 767 (42.1%)	(n=1779) 766 (43.1%)	0.98	[0.89, 1.08]	0.687
	Main secondary	567 (31.2%)	553 (31.1%)	1.01	[0.90, 1.14]	0.869
	All-cause death	368 (20.2%)	363 (20.4%)	1.00	[0.86, 1.15]	0.976
Double-blind + observational (max 9.5 yr; mean 8.7 yr)	Primary	(n=2605) 1118 (42.9%)	(n=2633) 1147 (43.6%)	0.95	[0.87, 1.03]	0.216
	Main secondary	829 (31.8%)	855 (32.5%)	0.95	[0.86, 1.05]	0.293
	All-cause death	545 (20.9%)	549 (20.9%)	0.98	[0.87, 1.11]	0.797

Primary endpoint = composite of all-cause death, MI, cardiac intervention, stroke, major leg amputation, leg revascularization; in PROactive, also included acute coronary syndrome.  
Main secondary endpoint = composite of all-cause death, MI, stroke.

Supported by: Takeda

1229

### Event rate assessment of myocardial infarction and stroke for pioglitazone relative to insulin in patients with type 2 diabetes mellitus

H. Liang<sup>1</sup>, A. Perez<sup>1</sup>, J. Yang<sup>2</sup>, C. Vallarino<sup>1</sup>, G. Fusco<sup>1</sup>, M. Bron<sup>2</sup>, V. Harikrishnan<sup>2</sup>, G. Joseph<sup>1</sup>, S. Yu<sup>2</sup>

<sup>1</sup>Takeda Global Research & Development Center, Inc., <sup>2</sup>Takeda Pharmaceuticals International, Inc., Deerfield, USA.

**Background and aims:** There is a paucity of data on the risk of cardiovascular (CV) events in patients with type 2 diabetes mellitus (T2DM) receiving pioglitazone (PIO) compared with insulin (INS). In this retrospective cohort study, the event rates of myocardial infarction (MI) and stroke in new users of PIO and INS were assessed.

**Materials and methods:** Data were extracted from May 1, 2000 to June 30, 2010 from the i3 InVision Data Mart, a US medical and prescription records database representing approximately 47 million covered lives during the period of analysis. Key outcomes were incident cases of a composite of MI and stroke requiring hospitalisation. A composite endpoint of all-cause mortality, MI and stroke was also created for a pre-planned additional analysis using death records obtained from the US Social Security Administration in March 2012. Kaplan-Meier curves were generated for these outcomes with adjustment of inverse probability weights derived from propensity scores. Hazard ratios (HR) for PIO versus INS and 95% confidence intervals (CIs) were estimated using Cox proportional hazards models including all patients, with inverse probability weights derived from propensity scores.

**Results:** A total of 56,536 type 2 diabetes patients (PIO: 38,588; INS: 17,948) aged  $\geq 45$  years were selected (mean age: 58.1 and 59.7 years, male gender: 59.6% and 53.0%, mean follow-up: 2.2 and 1.9 years, for PIO and INS, respectively). For the composite of MI and stroke, the incidence rate was lower for PIO (717 vs 2067 per 100,000 patient-years); the HR for PIO vs INS was 0.44 (95% CI [0.39, 0.50],  $p<0.0001$ ) (Table). Over the first 6 years, incidence rates of the composite of MI and stroke were higher in the INS group than in the PIO group. When compared individually, the risks of MI and stroke were both significantly lower in the PIO group: the HR for PIO versus INS for MI was 0.49 (95% CI [0.41, 0.57],  $p<0.0001$ ), and for stroke was 0.37 (95% CI [0.31, 0.45],  $p<0.0001$ ). In the analyses by gender, age, congestive heart failure, pre-index antidiabetic and lipid-altering medication use, PIO lowered the risk of the composite of MI and stroke in every subgroup compared with INS. Based on the additional analysis, the risk of all-cause mortality plus MI



or stroke was lower in the PIO group than in the INS group; HR for PIO versus INS was 0.37 (95% CI [0.35, 0.40],  $p < 0.0001$ ).

**Conclusion:** The results indicate that PIO was associated with significantly lower risk of MI and stroke requiring hospitalisation compared with INS. The results were similar when all-cause mortality was added as a component of the composite endpoint in the analysis.

Comparison of Composite Endpoints between PIO and INS			
Endpoint	HR (PIO vs INS)	95% CI	P Value
MI/Stroke	0.44	[0.39, 0.50]	<0.0001
All-cause mortality/MI/Stroke	0.37	[0.35, 0.40]	<0.0001

Supported by: Takeda Pharmaceuticals International, Inc.

## 1230

### MACE associated with pioglitazone: a meta-analysis of randomised, controlled trials

A. Perez, E. Song, A. Edmonds;

Takeda Global Research & Development - US, Deerfield, USA.

**Background and aims:** The benefit of pioglitazone (PIO) with respect to major adverse cardiovascular (CV) events (MACE) has been shown in previous studies.

**Materials and methods:** This analysis evaluated risk of MACE (primary; composite of CV death and nonfatal myocardial infarction or stroke) and serious CHF (secondary) in patients with type 2 diabetes mellitus (T2DM) receiving PIO ( $n=12,506$ ) vs placebo or active control ( $n=10,212$ ) in all 36 randomized controlled trials in the PIO clinical database. Treatment durations were 4 months to 4 years. Events were identified by searching preferred terms of adverse events identified through standard safety monitoring; in 3 studies, pre-specified central adjudication was performed. The primary endpoint was time from first dose to first event; hazard ratios (HRs) and 95% CIs for events on PIO vs comparator (COMP) were calculated using a Cox proportional-hazards model stratified by study with treatment as the independent variable. Kaplan-Meier estimates were also produced.

**Results:** The primary analysis showed a statistically significant reduction in risk of MACE with PIO vs COMP (HR, 0.82; Table), and Kaplan-Meier plots showed shorter time to first event with COMP. Secondary analyses as well as sensitivity analyses (also in the Table) showed similar results. The analysis also confirmed the known CHF risk of pioglitazone (HR for CHF causing hospitalization, 1.41; 95% CI, 1.15–1.74), with no corresponding increase in mortality.

**Conclusion:** This analysis supports the CV safety of PIO with respect to standard MACE endpoints; PIO consistently showed favorable CV outcomes compared with placebo and active control in T2DM, independent of background CV risk.

Analysis	Study Set	N	HR (95% CI) for MACE (PIO vs COMP)
Primary analysis	All controlled studies	22718	0.82 (0.71, 0.95)
	Double-blind controlled studies	22131	0.82 (0.71, 0.95)
Secondary analyses	Controlled studies excluding PROactive	17480	0.82 (0.62, 1.09)
	PROactive study	5238	0.82 (0.70, 0.97)
Sensitivity analyses (all controlled studies)	Excluding events >30 days after last dose	22718	0.83 (0.72, 0.97)
	Excluding studies with no MACE	20569	0.82 (0.71, 0.95)

Supported by: Takeda Global Research and Development

## 1231

### An assessment of all-cause mortality for pioglitazone compared with insulin in patients with type 2 diabetes mellitus

S. Yu<sup>1</sup>, J. Yang<sup>1</sup>, M. Bron<sup>1</sup>, A. Perez<sup>2</sup>, C. Vallarino<sup>2</sup>, G. Fusco<sup>2</sup>, H. Liang<sup>2</sup>, V. Harikrishnan<sup>1</sup>, G. Joseph<sup>2</sup>;

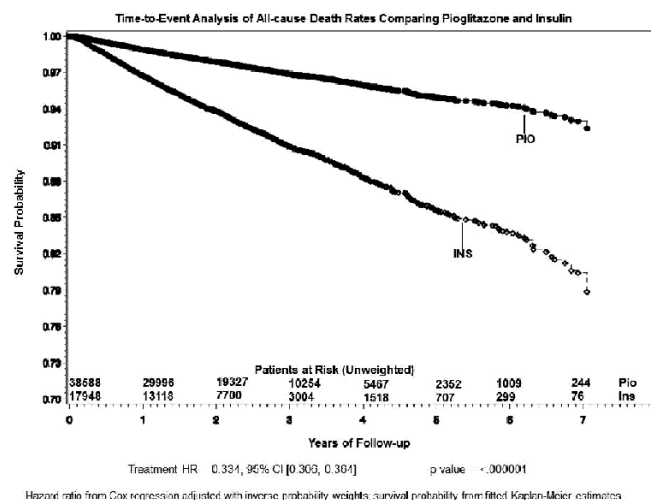
<sup>1</sup>Takeda Pharmaceuticals International, Inc., <sup>2</sup>Takeda Global Research & Development Center, Inc., Deerfield, USA.

**Background and aims:** Recent studies have examined the effects of antidiabetic agents on all-cause mortality in patients with type 2 diabetes mellitus (T2DM), with the data showing mixed results. This assessment explored all-cause mortality in new users of pioglitazone (PIO) or insulin (INS) for treatment of T2DM.

**Materials and methods:** We conducted a retrospective study of patients initiating PIO or INS treatment which extracted data from May 1, 2000 to June 30, 2010 from the i3 InVision Data Mart, a US claims database representing approximately 47 million covered lives during the period of analysis. The study's first analysis focused on cardiovascular, cancer, and bone endpoints. As a preplanned Stage II assessment, the mortality of the studied patient cohort was analysed with the patient data linked to death records obtained from the US Social Security Administration (SSA) in March 2012. A death record was considered a valid and non-censored event in the analyses if it occurred between the date of last medication/treatment/diagnosis observed in the i3 InVision Data Mart minus 30 days, and the date of the last day of insurance eligibility plus 60 days or June 30, 2010 (the study end date), whichever was first. Incident cases of death were confirmed by the SSA by matching a number of patient parameters: social security number, date of birth, last name, first name, and middle initial. Kaplan-Meier curves were generated for the occurrence of deaths in the PIO and INS groups with adjustment of inverse probability weights derived from propensity scores. Hazard ratios (HR) for PIO vs. INS and 95% confidence intervals (CIs) were estimated from Cox proportional hazards models including all available patients, with inverse probability weights derived from propensity scores.

**Results:** A total of 56,536 T2DM patients (PIO: 38,588; INS: 17,948) aged  $\geq 45$  years were selected from the i3 InVision Data Mart (mean age: 58.1 and 59.7 years, male gender: 59.6% and 53.0%, mean follow-up: 2.2 and 1.9 years, for PIO and INS; respectively). The total number of deaths identified was 2268 (PIO: 670; INS: 1598). The Kaplan-Meier curves for PIO and INS are shown in the attached figure. The risk of all-cause mortality was significantly lower in the PIO group compared with the INS group; HR for PIO vs INS was 0.334 (95% CI [0.306, 0.364],  $p < 0.0001$ ). In a subgroup analysis by gender, age, congestive heart failure, pre-index antidiabetic and lipid-altering medication use, all-cause mortality was significantly lower in the PIO group compared with the INS group in every subgroup analysed.

**Conclusion:** In this analysis, the PIO-initiating group had significantly lower risk of all-cause mortality compared with the INS-initiating group.



Supported by: Takeda Pharmaceuticals International, Inc.

## 1232

### A comparative study with metformin and pioglitazone versus metformin alone in nonalcoholic fatty liver disease in newly detected glucose intolerant patients

S.S. Sultana, F. Amin, A. Rahman, F. Afsana;  
BIRDEM, Dhaka, Bangladesh.

**Background and aims:** Non Alcoholic Fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome. The pathogenesis of steatosis and cellular injury is thought to be related mostly to insulin resistance. Insulin sensitizing drugs showed promising results in number of trials.

**Material and methods:** This is an open label clinical trial in newly detected Diabetes Mellitus (DM) and Impaired Glucose Intolerance (IGT) patients with raised ALT and NAFLD, to evaluate the effectiveness and superiority of pioglitazone and metformin combination to metformin alone. Forty nine patients with newly detected abnormal glucose tolerance, naïve to any anti-diabetic drug, were randomly selected, from the gastroenterology out-patient department BIRDEM, with the findings of ultasonogram changes of fatty liver and raised ALT and assigned to 6 months treatment with pioglitazone 30 mg plus metformin 1700 mg daily (Group 1, n=27) or only metformin 1700 mg alone (Group 2, n=22).

**Results:** Mean ( $\pm$ SD) age (yrs) of the study population was 45.80 $\pm$ 8.54, male female distribution of the study subjects were 65.3% and 34.7% respectively. Significant reduction of ALT, F, ABF, HbA1c, cholesterol, triglycerides of the study population were achieved either by metformin alone or with combination after six months [ (visit 1 vs visit 3, Mean $\pm$ SD) ALT: 97.99 $\pm$ 22. vs 55 $\pm$ 17.49 u/L, (p= 0.000); F : 9.1 $\pm$  1.9 vs 6.64 $\pm$ 0.94, mmol/L, (p=0.000); ABF: 13.3 $\pm$ 2.5 vs 8.69 $\pm$ 1.21 mmol/L, (p=0.000); HbA1c: 8.1 $\pm$ 0.9 vs 6.87 $\pm$ 0.57 % (p =0.000); CHOL: 205.9 $\pm$ 30.1 vs 186.12 $\pm$ 22.26 mg/dl, (p = 0.000); TG: 230.4 $\pm$ 48.1 vs 166.2 $\pm$ 31.82 (p = 0.000)]. In comparison between two groups, Group 1 had found to be significantly better glycemic control compared to their counterpart at the end of 6 months [( Group 1 vs Group 2, g, Mean $\pm$ SD) FBG: 6.37 $\pm$ 0.56 vs 6.98 $\pm$ 1.2; (p=0.024); ABF: 8.34 $\pm$ 0.84 vs 9.1 $\pm$ 1.46; (p=0.022);], serum cholesterol, TG, and ALT levels were also found to be significant change [ ( Mean  $\pm$  SD ) CHOL: 178.89 $\pm$ 18.59 vs 195 $\pm$ 23.55 mg/dl, (p = 0.010); TG: 155.85 $\pm$ 20.99 vs 178.91 $\pm$ 38.24 mg/ dl, (p =0.010); ALT: 55 $\pm$ 17.49 vs 45.74 $\pm$ 12.63 u/L, (p = 0.000);]. USG finding also found to be significantly reverse to normal from fatty changes in patients (77.7%) with both metformin and pioglitazone group than (18.8%) patients on metformin alone (p = 0.00) which all were fatty changes initially.

**Conclusion:** Treatment of NAFLD in newly detected Type 2 DM or IGT patients with high ALT by both metformin and pioglitazone is more effective in reduction of ALT and lipids and also able to reverse the severity of fatty changes of liver towards normal significantly.

## 1233

### Low cardiovascular (CV) risk hazard ratio observed with linagliptin in type 2 diabetes: further insights from a pre-defined CV meta-analysis

D. Neubacher<sup>1</sup>, O.-E. Johansen<sup>2</sup>, M. von Eynatten<sup>2</sup>, S. Patel<sup>3</sup>, H.-J. Woerle<sup>2</sup>;  
<sup>1</sup>Boehringer Ingelheim, Biberach, Germany, <sup>2</sup>Boehringer Ingelheim, Ingelheim, Germany, <sup>3</sup>Boehringer Ingelheim, Bracknell, UK.

**Background and aims:** Incidence of cardiovascular (CV) events is increased in type 2 diabetes (T2D) but the potential for CV risk modulation with glucose lowering is debated. Linagliptin, a DPP-4 inhibitor, provides a meaningful reduction in hyperglycaemia and also may have potential CV benefits. To further explore potential mechanisms behind this, we report additional relevant data from a large CV meta-analysis of linagliptin trial data.

**Materials and methods:** In this pre-specified meta-analysis from 8 Phase 3 randomised, controlled trials, CV events were prospectively adjudicated by a blinded independent expert committee. A composite primary endpoint of CV death, MI, stroke, and hospitalisation for unstable angina pectoris (UAP) was used. Results are given as hazard ratio (HR) for time to first occurrence of any components of the primary endpoint using a Cox regression model for linagliptin vs total comparators with factors of study (grouped by design), treatment (linagliptin vs combined comparators), gender (female vs male), race (Asian vs White), and time since T2D diagnosis (>5 vs  $\leq$ 5 yr). Comparative data for conventional CV risk factors across treatment groups are reported descriptively.

**Results:** 3319 patients received linagliptin once daily (5 mg: 3159, 10 mg: 160) and 1920 comparator (placebo: 977, glimepiride: 781, voglibose: 162). The overall study cohort (N=5239) seemed representative of a general T2D population among which overall HR was higher in patients with longer dura-

tion of T2D (1.51 [95% CI: 0.75–3.04]) and reduced in females (0.36 [95% CI: 0.16–0.84]) and Asians (0.28 [95% CI: 0.10–0.82]). A significantly reduced risk for the primary endpoint after treatment with linagliptin was indicated by the HR (0.36 [95% CI: 0.17–0.74]). Between-group changes in cholesterol and systolic and diastolic blood pressure were similar for linagliptin- and comparator-treated patients. Linagliptin treatment was associated with a greater reduction in HbA1c ( $-0.6 \pm 0.9\%$ ), fasting triglycerides ( $-11 \pm 136$  mg/dL), and no weight gain ( $0.01 \pm 3.09$  kg) in contrast to the total comparators (respectively  $-0.3 \pm 1.0\%$ ,  $-6 \pm 156$  mg/dL, and  $0.6 \pm 3.42$  kg).

**Conclusion:** Additional Cox regression analyses support the CV safety profile of linagliptin in a large phase III CV meta-analysis. The low CV HR observed with linagliptin cannot be fully explained by classical CV risk factors such as blood pressure and lipids. Further clinical outcome and mechanistic studies are underway to provide additional insights.

Supported by: *Boehringer Ingelheim*

## 1234

### Zofenopril and ramipril plus ASA in post-myocardial infarction patients with left ventricular systolic dysfunction: a SMILE-4 Study retrospective analysis in diabetics

C. Borghi, E. Ambrosioni;  
Internal Medicine, Policlinico S. Orsola, Bologna, Italy.

**Background and aims:** Combination of an angiotensin-converting enzyme inhibitor (ACEI) and acetyl salicylic acid (ASA) is a common option in patients with both heart failure and ischemic heart disease. However, the well-known pharmacological interaction between ACEI and ASA may be responsible for a reduction of the positive effect of ACEI on cardiovascular outcomes. In the SMILE-4 Study we have shown a more favorable impact of zofenopril + ASA than ramipril + ASA combination on 1-year occurrence of major cardiovascular events in patients with acute myocardial infarction (AMI) complicated by left ventricular dysfunction (LVD). The objective was to compare zofenopril and ramipril efficacy in combination with ASA in a subgroup of patients of the SMILE-4 with and without a history of diabetes mellitus at the time of enrolment in the study.

**Materials and methods:** The SMILE-4 was a phase IIIb, randomized, double-blind, parallel-group, multicenter, European study comparing the safety and efficacy of zofenopril 60 mg/day and ramipril 10 mg/day plus ASA 100 mg/day, in patients with LVD (clinical signs of heart failure or a left ventricular ejection fraction or LVEF <45%) following AMI. The primary study endpoint was 1-year combined occurrence of death or hospitalization for cardiovascular causes. Information diabetes at baseline was available in 693 out of the 716 patients of the intention-to-treat population.

**Results:** In the main study population the primary outcome was significantly reduced by zofenopril vs. ramipril (odds ratio, OR and 95% confidence interval, CI: 0.70, 0.51–0.96; p=0.028). Overall, 131 (18.9%) patients suffered from and 562 (81.0%) were free from diabetes mellitus. The rate of major cardiovascular events was slightly, but not significantly, lower under zofenopril than under ramipril in diabetics (30.9% vs. 41.3%; OR: 0.64, 0.31–1.30; p=0.216). In the group of patients without diabetes the hazard reduction was almost significantly in favor of zofenopril (27.9% vs. 34.9% ramipril; OR: 0.72, 0.50–1.03; p=0.072). The reduction in the risk of major cardiovascular events was similar in patients with and without diabetes (p=0.925).

**Conclusion:** This retrospective analysis of the SMILE-4 Study confirmed the good efficacy of zofenopril plus ASA in the prevention of long-term cardiovascular outcomes also in the subgroup of patients with diabetes mellitus.

Clinical Trial Registration Number: EudraCT 2004-001150-88

Supported by: *Menarini International, Laboratori Guidotti, Istituto Lusofarmaco d'Italia*

## 1235

### Effects of extended-release niacin/laropiprant (ERN/LRPT) on apolipoprotein (apo) B, LDL-cholesterol, and non-HDL-cholesterol targets in patients with type 2 diabetes

Y. Mitchell<sup>1</sup>, E. Brinton<sup>2</sup>, J. Triscari<sup>1</sup>, E. Chen<sup>1</sup>, A.O. Johnson-Levonas<sup>1</sup>, R. Ruck<sup>1</sup>, A. MacLean<sup>1</sup>, D. Maccubbin<sup>1</sup>;

<sup>1</sup>Merck Sharp & Dohme, Whitehouse Station, <sup>2</sup>Utah Foundation for Biomedical Research, Salt Lake City, USA.

**Background and aims:** ApoB is a good predictor of coronary risk especially in patients with type 2 diabetes (T2DM) and/or high triglycerides (TGs).

**Materials and methods:** This post-hoc analysis of a 36-week study evaluated apoB:LDL-C and apoB:non-HDL-C correlations in T2DM patients (n=796) randomized (4:3) to double-blind ERN/LRPT or placebo. After 4 weeks of treatment with 1 g/d, the ERN/LRPT dose was doubled to 2 g/d through study end. Patients had LDL-C  $\geq 1.55$  and  $< 2.97$  mmol/L and TG  $< 5.65$  mmol/L following a 4-week lipid-modifying run-in. Approximately 78% of patients were taking statins at baseline. Simple linear regression analyses calculated LDL-C and non-HDL-C levels corresponding to apoB value of 0.9 g/L at baseline and Week 12 in the overall population and patients with or without high TG (ie, baseline TG  $<$  and  $\geq 2.26$  mmol/L).

**Results:** LDL-C and non-HDL-C were well correlated with apoB at baseline and Week 12 in both treatment groups. At baseline and Week 12, predicted LDL-C and non-HDL-C values were lower than the minimum recommended goals of 2.59 and 3.36 mmol/L, respectively. When analyses were examined by baseline TG, predicted LDL-C values were significantly lower in high TG vs low TG patients (95% CIs did not overlap) at baseline and Week 12 for both treatment groups. Predicted non-HDL-C values were significantly higher in high TG vs low TG patients at baseline but not Week 12.

**Conclusions:** Irrespective of treatment with ERN/LRPT or placebo, more intensive LDL-C and non-HDL-C goals should be achieved in T2DM pts with and without hypertriglyceridemia to reach an apoB target  $\leq 0.9$  g/L.

Treatment Group	N*	apoB:LDL-C Pearson Correlation Coefficient (95% CI)	Predicted LDL-C (mmol/L) at apoB=0.9 g/L (95% CI)	apoB:non-HDL-C Pearson Correlation Coefficient (95% CI)	Predicted non-HDL-C (mmol/L) at apoB=0.9 g/L (95% CI)
<b>Baseline (ie, pre-randomization measurement)</b>					
ERN/LRPT	395	0.73 (0.68, 0.77)	2.19 (2.15, 2.23)	0.87 (0.85, 0.89)	2.90 (2.87, 2.94)
TG $< 2.26$ mmol/L	322	0.80 (0.75, 0.83)	2.22 (2.18, 2.26)	0.86 (0.83, 0.89)	2.86 (2.83, 2.89)
TG $\geq 2.26$ mmol/L	73	0.74 (0.61, 0.83)	1.85 (1.73, 1.98)	0.81 (0.71, 0.87)	3.20 (3.10, 3.31)
Placebo	326	0.72 (0.66, 0.76)	2.18 (2.14, 2.21)	0.87 (0.84, 0.89)	2.87 (2.84, 2.90)
TG $< 2.26$ mmol/L	280	0.72 (0.66, 0.78)	2.22 (2.18, 2.26)	0.82 (0.78, 0.86)	2.84 (2.81, 2.88)
TG $\geq 2.26$ mmol/L	46	0.85 (0.73, 0.91)	1.84 (1.71, 1.97)	0.91 (0.83, 0.95)	3.05 (2.94, 3.15)
<b>Week 12 (ie, last post-baseline measurement)</b>					
ERN/LRPT	394	0.82 (0.79, 0.85)	2.11 (2.07, 2.15)	0.89 (0.87, 0.91)	2.82 (2.78, 2.86)
TG $< 2.26$ mmol/L	321	0.84 (0.80, 0.87)	2.20 (2.15, 2.24)	0.87 (0.84, 0.90)	2.78 (2.74, 2.83)
TG $\geq 2.26$ mmol/L	73	0.86 (0.78, 0.91)	1.93 (1.86, 2.00)	0.88 (0.82, 0.92)	2.89 (2.78, 3.00)
Placebo	323	0.73 (0.68, 0.78)	2.14 (2.10, 2.18)	0.87 (0.84, 0.90)	2.84 (2.80, 2.88)
TG $< 2.26$ mmol/L	279	0.77 (0.71, 0.81)	2.17 (2.13, 2.21)	0.85 (0.82, 0.88)	2.82 (2.79, 2.86)
TG $\geq 2.26$ mmol/L	44	0.69 (0.49, 0.82)	1.95 (1.88, 2.01)	0.89 (0.79, 0.93)	2.91 (2.75, 3.07)

\*Number of patients with paired apoB and LDL-C as well as apoB and non-HDL-C measurements

with LDL-C  $\geq 70$  and  $\leq 160$  mg/dl. The percent change in LDL-C and other lipids was estimated within each subgroup separately using a constrained longitudinal data analysis model with terms for treatment, time, time-by-treatment interaction, stratum, and time-by-stratum interaction. Safety was also assessed.

**Results:** In obese subjects, percent changes in LDL-C and other lipids were greater with EZ/Simva compared with doubling the baseline statin dose or switching to rosuvastatin, except HDL-C and Apo A-I. In non-obese subjects, percent changes in LDL-C, total cholesterol non-HDL-C, Apo B and Apo A-I were greater with EZ/Simva compared with doubling the baseline statin dose or switching to rosuvastatin. Also in non-obese subjects, treatment with EZ/Simva resulted in greater reductions in triglycerides compared with rosuvastatin and greater changes in HDL-C vs doubling the baseline statin dose. (Table) There were no clinically significant safety concerns in any of the treatment subgroups.

**Conclusion:** Regardless of baseline BMI, in this population of high risk diabetic subjects, switching to ezetimibe/simvastatin 10/20 was more effective at reducing LDL-C, total cholesterol and Apo B compared with doubling the baseline statin dose to simvastatin 40 mg or atorvastatin 20 mg or switching to rosuvastatin 10 mg. The safety and tolerability profiles were generally similar between treatment groups.

Table. LS mean % change in lipids								
		LDL-C	TC	TG	HDL-C	non-HDL-C	Apo B	Apo A-I
non-Obese	EZ/Simva (n=136)	-25.2	-13.9	-4.8	1.8	-19.9	-16.6	2.0
	Doubling Statin (n=69)	-4.9	-4.1	-5.3	-0.2	-4.8	-5.6	-2.3
	Rosuva 10 (n=126)	-17.4	-9.4	-2.0	2.6	-13.9	-11.6	1.3
Obese	EZ/Simva (n=178)	-21.6	-12.7	-6.1	1.2	-17.3	-13.7	-0.4
	Doubling Statin (n=90)	-10.7	-5.3	-0.8	1.8	-8.2	-8.0	0.0
	Rosuva 10 (n=189)	-20.7	-11.4	-4.2	1.6	-16.1	-12.3	0.6

Clinical Trial Registration Number: NCT00862251

Clinical Trial Registration Number: NCT00485758

Supported by: Merck Sharp & Dohme Corp.

## 1236

### Consistency of effect of ezetimibe/simvastatin compared with intensified lipid-lowering treatment strategies in obese and non-obese diabetic subjects

J.B. Rosen<sup>1</sup>, J.G. Jimenez<sup>2</sup>, V. Pirags<sup>3</sup>, H. Vides<sup>4</sup>, R. Massaad<sup>5</sup>, M.E. Hanson<sup>6</sup>, P. Brudi<sup>6</sup>, J. Triscari<sup>6</sup>

<sup>1</sup>Clinical Research of South Florida, Coral Gables, USA, <sup>2</sup>Hospital CIMA San Jose, Escazu, Costa Rica, <sup>3</sup>University of Latvia, Riga, Latvia, <sup>4</sup>Viljandi Hospital, Vildjandimaa, Estonia, <sup>5</sup>MSD Belgium, Brussels, Belgium, <sup>6</sup>Merck Sharp & Dohme Corp., Whitehouse Station, USA.

**Background and aims:** Diabetes is commonly associated with the presence of obesity and both conditions increase the risk of cardiovascular disease (CVD) and CVD events. The objective of this analysis was to assess the consistency of treatment effect of switching to ezetimibe/simvastatin 10/20 mg vs doubling the baseline statin dose (to simvastatin 40 mg or atorvastatin 20 mg) or switching to rosuvastatin 10 mg in subgroups of obese diabetic subjects (n=466) and non-obese diabetic subjects (n=342) based on body mass index (BMI)  $< 30$  kg/m<sup>2</sup> or  $> 30$  kg/m<sup>2</sup>.

**Materials and methods:** This was a post hoc analysis of a randomized, double-blind, 6-week study of adults 18-79 years with CVD and diabetes mellitus



## PS 109 Vascular mechanisms and biomarkers

### 1237

#### Arterial stiffness is negatively related to insulin sensitivity in young type 1 diabetic patients

I. Pietrzak, W. Fendler, B. Mianowska, A. Zmysłowska, W. Młynarski, A. Szadkowska;

Department of Pediatrics, Oncology, Hematology and Diabetology, Medical University of Lodz, Poland.

**Background and aims:** Complications are the main reason of premature death in patients with diabetes mellitus. Increased arterial stiffness is observed even in young diabetic patients. Insulin resistance is one of the most important risk factors for cardiovascular diseases. The aim of the study was to determine the relationship between ambulatory arterial stiffness index (AASI) value and insulin sensitivity adolescents with type 1 diabetes.

**Materials and methods:** Eighty eight patients (52 males), aged  $16.1 \pm 2.4$  years, with an average diabetes duration of  $4.6 \pm 3.9$  years and without any evidence of arterial hypertension were recruited. AASI value was determined based on 24-hour automatic blood pressure monitoring results. Euglycemic - hyperinsulinemic clamp was performed to estimate insulin sensitivity. Glucose disposal rate (M value) determined during the last 30 min of the test was calculated as a surrogate of insulin resistance. Height, weight, waist circumference were measured and body mass index (BMI) were calculated. HbA1c and lipids were measured.

**Results:** Mean AASI equalled  $0.24 \pm 0.19$ . In univariate analysis AASI showed significant, positive correlation with BMI-Z score ( $r=0.23$ ,  $p=0.034$ ) and negative correlation with M value ( $r=-0.31$ ,  $p=0.005$ ). No correlations between AASI and patients' sex, diabetes duration, waist-Z score and HbA1c were found. Positive correlations with age and LDL cholesterol level were noted but did not reach statistical significance. Multivariate regression analysis showed that M value was the only factor which significantly correlated with AASI values.

**Conclusion:** In young type 1 diabetic patients arterial stiffness is negatively related to insulin sensitivity. Thus, insulin resistance may be considered as a risk factor for early macrovascular complications in type 1 diabetic patients.

*Supported by: Ministry of Science and Higher Education NN 407 1878 36, NN 407 2851 39*

### 1238

#### Metabolic syndrome and related components predict increased arterial stiffness (AS) in youth with type 1 diabetes: the SEARCH CVD study

D. Dabelea<sup>1</sup>, J. Talton<sup>2</sup>, R.F. Hamman<sup>1</sup>, R. D'Agostino Jr.<sup>2</sup>, R.P. Wadwa<sup>3</sup>, E. Urbina<sup>4</sup>, L. Dolan<sup>5</sup>, S. Daniels<sup>6</sup>, S. Marcovina<sup>7</sup>;

<sup>1</sup>Epidemiology, Colorado School of Public Health, Aurora, <sup>2</sup>Wake Forest School of Medicine, Winston Salem, <sup>3</sup>Pediatrics, Barbara Davis Center, Aurora, <sup>4</sup>Cincinnati Children's Hospital, <sup>5</sup>Pediatrics, Cincinnati Children's Hospital, <sup>6</sup>Pediatrics, University of Colorado School of Medicine, Aurora, <sup>7</sup>Medicine, University of Washington, Seattle, USA.

**Background and aims:** Adults with childhood onset type 1 diabetes (T1D) are at increased risk of premature cardiovascular disease (CVD). Yet, the natural history, the risk factors for premature CVD, and the progression from preclinical to advanced disease have not been studied systematically. In adults, markers of sub-clinical CVD such as increased pulse wave velocity (PWV) were shown to predict all cause and CVD mortality. We explored the rate of change and the role of CVD risk factors in predicting changes in PWV, a measure of arterial stiffness (AS), in youth with T1D, free of clinically diagnosed CVD.

**Materials and methods:** SEARCH CVD is an ancillary study to the SEARCH for Diabetes in Youth, a multicenter study that conducts population-based ascertainment of physician diagnosed diabetes in youth. PWV (meters/sec) in the carotid-femoral segment was measured with the SphygmoCor device. Higher PWV indicates increased central AS. CVD risk factors [triglycerides (TG), HDL-, LDL-cholesterol, waist circumference (WC), blood pressure, albumin/creatinine ratio (ACR), hemoglobin A1c (A1c) and metabolic syndrome (MetS: at least 2 risk factors, including high waist, TG, BP, low HDL-c)] were measured using standard protocols. Linear mixed models were used to model the rate of change in log-transformed PWV over time and explore significant predictors.

**Results:** There were 298 youth with T1D who had repeated measures of PWV and CVD risk factors approximately 5 years apart. At baseline, mean age was 14.5 yrs (SD 2.8), duration was 4.8 yrs (SD 3.8), and 87.6% were non-Hispanic white. At follow-up, average duration of T1D was 10.1 (SD 3.9) years. Over the follow-up period, PWV increased from 5.2 to 5.9 m/sec, an average annual change of 2.8% ( $p<0.0001$ ). In models adjusted for age, sex, and race/ethnicity, the presence of MetS at baseline was associated with a 1.07% higher increase in PWV ( $p=0.0035$ ), indicating a significantly faster stiffening of central arteries in youth with T1D and MetS, as compared to those without MetS. Individual components of the MetS, specifically WC ( $p<0.0001$ ), TG levels ( $p=0.029$ ) systolic ( $p<0.0001$ ) and diastolic BP z-scores ( $p<0.0001$ ), were also associated with increase in PWV. The effects were similar across the entire distribution of these CVD risk factors. In contrast, baseline HDL-c and LDL-c, ACR and A1c did not predict change in PWV. Moreover, an increase in WC ( $p<0.0001$ ), LDL-c ( $p=0.0095$ ) and TG levels ( $p=0.048$ ) over time were also associated with higher rate of change in PWV.

**Conclusion:** Our results show that youth with T1D and relatively short duration of diabetes have a significant increase in PWV over only 5 years. Nevertheless, the impact of this increase on later CVD morbidity and mortality is not currently known. The increase in PWV was predicted by presence of MetS and related components, especially central obesity and dyslipidemia. It is important to learn whether reducing MetS and its' components early in life will slow the progression of AS and reduce the burden of CVD in people with T1D.

*Supported by: NIH R01 DK078542*

### 1239

#### Activated complement C3 (C3a) is cross-sectionally associated with carotid intima-media thickness and inversely with the ankle-arm blood pressure index: the CODAM study

E. Hertle<sup>1</sup>, M.M. van Greevenbroek<sup>1</sup>, C.J. van der Kallen<sup>1</sup>, S.L. Geijselaers<sup>1</sup>, I. Ferreira<sup>1,2</sup>, E.J. Feskens<sup>3</sup>, C.G. Schalkwijk<sup>1</sup>, C.D. Stehouwer<sup>1</sup>;

<sup>1</sup>CARIM School for Cardiovascular Diseases and Dept. of Internal Medicine, Maastricht University, <sup>2</sup>Dept. of Clinical Epidemiology and Medical Technology Assessment (KEMTA), Maastricht University Medical Centre, <sup>3</sup>Division of Human Nutrition, Section Nutrition and Epidemiology, Wageningen University, Netherlands.

**Background and aims:** Macrovascular atherosclerotic disease is a serious diabetic complication that involves inflammatory and immunological pathomechanisms. The central complement component C3 has been implicated in the development of metabolic and cardiovascular disease (CVD). C3a, an anaphylatoxin generated upon C3 activation, can induce multiple proinflammatory effects and may, as such, contribute to atherosclerosis. The study aim was to investigate the association of C3a with prevalent CVD and subclinical markers of atherosclerosis, i.e. carotid intima-media thickness (cIMT) and ankle-arm blood pressure index (AAIx) in a cohort of individuals at high-risk for type 2 diabetes (DM2) and CVD.

**Materials and methods:** The conducted cross-sectional analyses among 521 individuals (61% men, mean $\pm$ SD age of  $59.5 \pm 6.9$  years, 23% with impaired glucose metabolism and 23% with DM2) from the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) Study. Prevalent CVD was defined on the basis of self-reports, signs of myocardial infarction on an ECG and an AAIx $<0.9$ ; cIMT was measured by ultrasonography and AAIx by Doppler ultrasound. Associations between C3a and study outcomes were analysed with multiple logistic or linear regression analyses and all analyses were adjusted first, for age and sex, and further for glucose metabolism status, waist, blood lipids, blood pressure, renal function, physical activity, smoking, use of medication and C3, the precursor of C3a. We included additional adjustments for HOMA2-IR, and low-grade inflammation (LGI; expressed as a score calculated from 6 inflammatory markers) to ascertain whether these could explain part of the observed associations.

**Results:** The prevalence of CVD was 27%, cIMT was  $0.77 \pm 0.15$  mm, AAIx was  $1.10 \pm 0.13$  and median [IQR] of plasma C3a was 59 [50-73] ng/ml. Plasma C3a (per doubling) was associated with CVD [age and sex adjusted OR=1.55 (95%CI: 0.99-2.40),  $p=0.053$ ], but this association was not independent of other covariates [OR=0.94 (0.55-1.61),  $p=0.821$ ]. In contrast, higher levels of C3a were significantly associated with higher cIMT [ $\beta=0.033$  mm, (95% CI: 0.004; 0.063),  $p=0.028$ ] and lower AAIx [ $\beta=-0.033$ , (-0.056; -0.010),  $p=0.004$ ]. The associations with AAIx but less so with cIMT were partly explained by LGI [-0.007 (95%CI: -0.015; -0.001) and 0.004 (-0.003; 0.012), respectively], but not by HOMA2-IR.

**Conclusion:** Plasma C3a was independently associated with higher cIMT and lower AAIx, both supporting a role of activated complement in atherosclerosis. The lack of association with CVD might be explained by the concept that CVD comprises not only atherosclerosis/stenosis but also atherothrombosis and hypofibrinolysis and therefore may represent atherosclerosis less specifically than cIMT or AAIx. The independent association with subclinical atherosclerosis but not with CVD suggests that C3a may be primarily involved in atherogenesis, but to a lesser extent in processes that result in plaque rupture and atherothrombotic events.

Supported by: NHS2010B194, NHS2006T050

## 1240

### Arterial elastic wall properties are impaired early in the development of insulin resistance

V.A. Lambadiari<sup>1</sup>, I. Ikonomidis<sup>2</sup>, F. Spanoudi<sup>1</sup>, F. Kousathana<sup>1</sup>, C. Koukoulis<sup>2</sup>, G. Matsagouras<sup>1</sup>, N. Kadoglou<sup>2</sup>, H. Triantafyllidis<sup>2</sup>, M. Varoudi<sup>2</sup>, V. Tritakis<sup>2</sup>, M. Anastasiou-Nana<sup>2</sup>, J. Lekakis<sup>2</sup>, G. Dimitriadis<sup>1</sup>;

<sup>1</sup>2nd Dpt of Internal Medicine, Research Institute and Diabetes Center, <sup>2</sup>2nd Cardiology Department, Attikon University Hospital, Athens University Medical School, Greece.

**Background and aims:** Studies link insulin resistance with increased arterial stiffness. Arterial stiffness is associated with increased risk for cardiovascular disease. We investigated whether first degree relatives of diabetic patients have similarly impaired arterial wall properties with patients with diabetes as assessed after an oral glucosetolerance test (OGTT).

**Materials and methods:** 60 subjects underwent a standard 75-gr oral glucose tolerance test (OGTT) and plasma glucose and insulin levels were measured at 0, 30, 60, 90 and 120min after glucose loading. At the same time intervals, the carotid-femoral pulse-wave velocity (PWVc) was measured, using the Complior apparatus and aortic PWV (PWVa) and augmentation index (AI), using an oscillometric method (Arteriograph). Insulin resistance was assessed: a) in the fasting state, using homeostatic model assessment (HOMA) and hepatic insulin sensitivity-Index (HIS) b) postchallenge, using Matsuda index and insulin sensitivity index (ISI 0-120).

**Results:** Of the 60 subjects 20 were first degree relatives of type 2 diabetic patients and had normal OGTT (relatives), 20 had normal OGTT and no family history of diabetes (controls), and 20 were classified as diabetic (DM). Compared to controls, DM and relatives had both higher baseline PWVc (10.4±2.6 vs. 9.1±1.3 vs 8±1.5 m/sec, p<0.05), PWVa (9.1±2 vs. 8.9±2 vs. 7.3±1.6 m/sec, p<0.05), AI (24.5±9 vs. 24.2±14, 18±15%, p<0.05), insulin (14±6 vs. 15±4 vs. 10±4 µU/ml, p<0.05), HOMA, (3.9±2.1 vs. 3.6±1.6 vs. 2.4±1, p<0.05) and lower HIS (0.32±0.24 vs. 0.33±0.24 vs. 0.48±0.18, p<0.05), ISI (50±24 vs. 73±22 vs 93±17 mg.L<sup>2</sup>/mmol/mU/min, p<0.05), Matsuda index (3.1±1.6 vs. 3.0±1.6 vs. 5.3±1.2, p<0.05). Age, sex and BMI were similar between subgroups (p=ns). Compared to baseline, AI was reduced at 30 min by 30% in controls and relatives (p<0.01) but only 6% in diabetics (p=0.5). Conversely compared to baseline, insulin levels were increased at 30 min, to 54 µU/ml (484%) in controls, 56 µU/ml (266%) in DM and 110 µU/ml (681 %) in relatives (p<0.01). In all subjects, the % decrease in AI at 30 min was related to the corresponding % increase in insulin levels (r=-0.46, p<0.05).

**Conclusion:** First degree relatives and diabetic subjects have increased arterial stiffness and abnormal wave reflection compared to those with normal OGTT and no family history of diabetes. Controls and relatives show an acute decrease in AI related to the corresponding increase in insulin level likely because of insulin stimulation of endothelial cell nitric oxide (NO) synthesis. However relatives succeed this effect by raising their insulin levels nearly twice as those of normal subjects suggesting the onset of insulin resistance as confirmed by estimated indices of insulin resistance. In diabetic subjects the effect of insulin on AI is blunted likely because of severe insulin resistance.

## 1241

### Association of pulse wave velocity with cardiac autonomic neuropathy in patients with type 2 diabetes

S. Chorepsima<sup>1</sup>, I. Moysakakis<sup>2</sup>, D. Papadogiannis<sup>1</sup>, A. Kokkinos<sup>1</sup>, A. Protogerou<sup>1</sup>, N. Katsilambros<sup>1</sup>, N. Tentolouris<sup>1</sup>;

<sup>1</sup>1st Department of Propaedeutic Medicine, <sup>2</sup>Department of Cardiology, Laiko General Hospital, Athens, Greece.

**Background and aims:** Arterial stiffness is significantly increased in diabetes, regardless of the presence or absence of complications of the disease. Pulse

wave velocity (PWV) is considered as the gold-standard measurement to assess arterial stiffness. Limited data exist on the relationship between PWV and cardiac autonomic function in patients with type 2 diabetes (T2D). The aim of this cross-sectional study was to examine the association between PWV and indices of cardiac autonomic nervous function in patients with T2D.

**Materials and methods:** A total of 194 subjects with T2D were studied. PWV of the carotid-femoral segment was determined by applanation tonometry. Patients were classified as having normal and abnormal PWV according to age-corrected published recommendations. Short-term analysis of the heart rate variability (HRV) was performed in all subjects using the computer-aided examination and evaluation system VariaCardio TF4, whereas baroreflex sensitivity (BRS) was assessed in all subjects by the spontaneous sequence method using the BaroCor System. In addition, demographic and anthropometric data were obtained and lipids, renal function and HbA1c were measured.

**Results:** Patients with normal PWV (n=145) and patients with abnormal (n=49) PWV did not differ significantly in terms of age, sex, duration of diabetes, HbA1c values, renal function, lipid profile, smoking habits and prevalence of macrovascular complications as well as of retinopathy and microalbuminuria, while peripheral neuropathy was more common in the group of subjects with abnormal PWV (p=0.016). Patients with abnormal PWV had higher mean blood pressure (MP) than those with normal PWV (p=0.003). The total power (TP) of the HRV and the power of the high frequency (PHF) were lower in patients with abnormal PWV [125.8 (48.3-426.8) vs. 273.2 (111.5-571.1) ms<sup>2</sup>, p=0.006 and 39.0 (16.0-165.4) vs. 115.1 (42.9-252.0) ms<sup>2</sup>, p=0.002, respectively]. The power of the low frequency (PLF) and the PLF/ PHF ratio were not different between the two groups, while all time domain parameters of HRV like NN (p=0.040), SDNN (p=0.029) and MSDD (p=0.003) were significantly lower in patients with abnormal PWV. BRS was not significantly different between participants with normal and abnormal PWV [5.76 (4.11-8.53) ms/mmHg vs. 4.88 (3.18-7.19), p=0.146]. Univariate logistic regression showed that higher MP, presence of retinopathy and peripheral neuropathy as well as lower log values of PLF, PHF, TP, NN, SDNN and MSDD were associated with higher odds of abnormal PWV. Moreover, no significant associations were found between age, gender, BRS, glycaemic control, lipid profile, macrovascular complications or smoking status with PWV. Multivariate analysis, after adjustment for the effect of age, gender, duration of diabetes and MP, demonstrated that the odds [OR (95% confidence intervals)] of abnormal PWV increased significantly with lower log values of PHF [2.32 (1.27-4.24), p= 0.006], PLF [1.77 (1.00-3.14), p= 0.050], TP [2.22 (1.17-4.23), p= 0.015] and MSDD [2.23 (1.22-4.06), p= 0.009], as well as with higher MP [1.04 (1.01-1.07), p=0.009].

**Conclusion:** Cardiac autonomic dysfunction, assessed by parameters of heart rate variability, is a significant and independent determinant of increased arterial stiffness in subjects with T2D.

## 1242

### Non-alcoholic fatty liver disease is associated with carotid intima-media thickness only in type 2 diabetic subjects with insulin resistance

Y.-W. Cho<sup>1</sup>, Y.-J. Choi<sup>2</sup>, S.-W. Park<sup>1</sup>, E.-J. Lee<sup>3</sup>, K.-B. Huh<sup>2</sup>;

<sup>1</sup>Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam, <sup>2</sup>Huh's Diabetes Center and the 21st Century Diabetes and Vascular Research Institute, Seoul, <sup>3</sup>Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.

**Background and aims:** Non-alcoholic fatty liver disease (NAFLD) is commonly associated with insulin resistance, obesity, metabolic syndrome, and diabetes. Although a lot of studies suggest that NAFLD is independently associated with an increased risk of atherosclerotic diseases, this association is less clear in type 2 diabetic subjects. The aim of the present study was to investigate whether there is a difference in carotid atherosclerotic burden in type 2 diabetic subjects according to NAFLD and/or insulin resistance.

**Materials and methods:** This was an observational study performed in 4437 patients with type 2 diabetes, consecutively enrolled in our Diabetes Center, who have never been treated with thiazolidinediones. Liver and carotid ultrasounds were performed on each subject. Carotid atherosclerosis was defined as having a clearly isolated focal plaque or mean carotid intima-media thickness (C-IMT) ≥ 1.1 mm. Insulin sensitivity was directly assessed by a rate constant for plasma glucose disappearance (Kitt) using short insulin tolerance test and insulin resistance was defined as Kitt < 2.5 %/min.

**Results:** Among subjects without NAFLD (n=1211), 747 subjects had insulin resistance. In contrast, among the subjects with NAFLD (n=3226), 747

patients did not have insulin resistance. Age, duration of diabetes, fasting glucose and HbA1c were longer or higher in those with insulin resistance. BMI and waist circumference were higher in those with NAFLD compared to those without NAFLD. There were significant differences in mean C-IMT and frequency of carotid atherosclerosis between groups classified by insulin resistance within the same NAFLD strata. Especially, mean C-IMT was the highest in subjects with both NAFLD and insulin resistance ( $0.844 \pm 0.004$  [mean  $\pm$  S.E.] mm vs.  $0.786 \pm 0.008$ ,  $0.821 \pm 0.007$ , and  $0.807 \pm 0.006$  mm,  $P$  for trend  $< 0.001$ , respectively, in insulin sensitive subjects without NAFLD, insulin resistant subjects without NAFLD, and insulin sensitive subjects with NAFLD). An augmentative effect of NAFLD and insulin resistance on mean C-IMT remained significant after adjusting for potential confounders; age, sex, duration of diabetes, various vascular diseases, hypoglycemic or anti-hypertensive agents, and BMI. However, mean C-IMT in subjects having either NAFLD or insulin resistance was not higher compared to that in those having neither NAFLD nor insulin resistance. And, the relationship between NAFLD and/or insulin resistance and the frequency of carotid atherosclerosis disappeared after adjustment.

**Conclusion:** In subjects with type 2 diabetes, having either NAFLD or insulin resistance is not associated with carotid atherosclerotic burden. However, having both NAFLD and insulin resistance seem to be a determinant factor associated with C-IMT.

## 1243

### Association between pulse pressure and cardiovascular risk in diabetes mellitus: a meta-analysis

C. Horikawa<sup>1</sup>, S. Kodama<sup>1</sup>, Y. Heianza<sup>1</sup>, S. Yoshizawa<sup>1</sup>, K. Fujihara<sup>1</sup>, S. Tanaka<sup>2</sup>, K.T. Iida<sup>3</sup>, Y. Yachi<sup>1</sup>, Y. Ohashi<sup>4</sup>, H. Sone<sup>1</sup>

<sup>1</sup>Department of Endocrinology and Metabolism, University of Tsukuba Mito Medical Center, Mito, <sup>2</sup>Department of Clinical Trial Design and Management, Kyoto University Hospital, <sup>3</sup>Department of Lifestyle Medicine, Ochanomizu University, Tokyo, <sup>4</sup>Department of Biostatistic, Epidemiology and Preventive Health Sciences, University of Tokyo, Japan.

**Background and aims:** It is important to determine cardiovascular (CV) risk in relation to pulse pressure (PP) especially in diabetes mellitus (DM) because patients with DM have more rigid arteries than those without DM. However, the quantitative association between PP and CV risk has been heterogeneous among studies. Our aim of this meta-analysis is to estimate the strength of the association between PP and CV risk and to determine the reason for this heterogeneity.

**Materials and methods:** Electronic literature (up to Feb. 25, 2012) search was conducted for cohort studies reporting relative risk (RR) of cardiovascular disease (CVD), coronary heart disease (CHD), or stroke in relation to PP in patients with DM. The most fully adjusted RR for these outcomes in each study for each +10 mmHg increase in PP in DM was pooled with a random-effects model.

**Results:** Thirteen articles were included in this meta-analysis. RR (95% confidence interval [95%CI]) for a +10 mmHg increase in PP was 1.12 (1.04–1.20) for CVD (12 studies) (Figure), 1.18 (1.08–1.29) for CHD (5 studies), and 1.13 (1.07–1.19) for stroke (4 studies). In studies that concurrently assessed CV risk in relation to both PP and other blood pressure (BP) indexes such as systolic BP (SBP) (7 studies), diastolic BP (DBP) (8 studies), or mean aortic pressure (MAP) (4 studies), the pooled RR of CVD for a +10 mmHg increase in PP vs. other BP indexes was 1.09 vs. 1.06 for PP vs. SBP, 1.12 vs. 1.04 for PP vs. DBP, and 1.18 vs. 1.23 for PP vs. MAP. Stratified analysis indicated that the CVD risk for an incremental increase in PP was attenuated in studies adjusted for duration of diabetes ( $P=0.03$ ) or indicators of nephropathy ( $P=0.001$ ).

**Conclusion:** Higher PP is associated with elevated CV risk in patients with DM. However, the elevated risk may be due to DM itself or DM-specific complications rather than high PP. Evidence is insufficient for measuring PP in addition to SBP in diabetic patients.

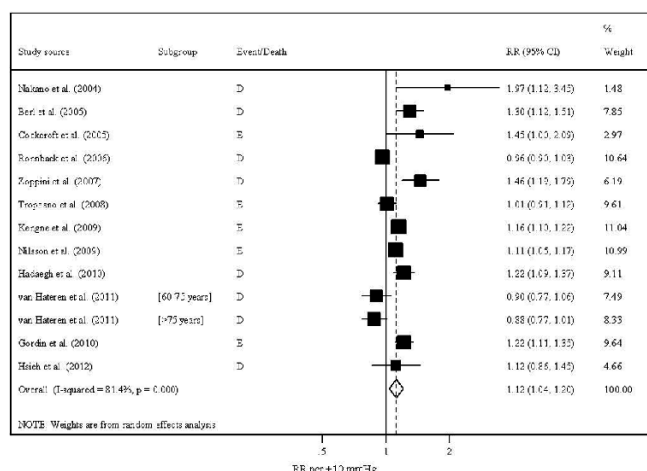


Figure Forest plot with 95% confidence interval (95% CI) regarding relative risk (RR) of cardiovascular disease (CVD) for a +10 mmHg increase in pulse pressure (PP). The pooled RR is indicated by a diamond. Area of each square is proportional to study weight (i.e., inverse of variance).

## 1244

### First ever 24-hour central blood pressure in patients with type 1 diabetes

S. Theilade<sup>1</sup>, M. Lajer<sup>1</sup>, C. Joergensen<sup>1</sup>, F. Persson<sup>1</sup>, G. Andresdottir<sup>1</sup>, H. Reinhard<sup>1</sup>, S.E. Nielsen<sup>1</sup>, P. Lacy<sup>2</sup>, B. Williams<sup>2</sup>, P. Rossing<sup>1</sup>

<sup>1</sup>Steno Diabetes Center, Gentofte, Denmark, <sup>2</sup>Department of Cardiovascular Sciences, University of Leicester, UK.

**Background and aims:** To investigate whether measurement of 24-hour ambulatory central aortic systolic pressure (CASP) using calibrated tonometry is feasible and associated with albuminuria in type 1 diabetes.

**Methods and materials:** Cross-sectional study, 649 Caucasian T1DM patients and 86 controls (C). Patients were 69 with short diabetes duration ( $<10$  years), normoalbuminuria ( $<30$ mg/24-hour) and not receiving antihypertensive treatment (SN); 211 with longstanding diabetes ( $\geq 10$  years) and normoalbuminuria (LN); 164 with microalbuminuria (30–300mg/24-hour) (Mi) and 205 with macroalbuminuria ( $>300$ mg/24-hour) (Ma). 24-hour ambulatory blood pressures were measured with tonometry by BPro (HealthStats, Singapore), previously validated according to ESH and AAMI. Dipping was percentage decrease in blood pressure (BP) from day- to nighttime (12pm–6am).

**Results:** For the groups (C, SN, LN, Mi, Ma): mean  $\pm$  SD age was  $49 \pm 12$ ;  $39 \pm 13$ ;  $57 \pm 11$ ;  $58 \pm 11$  and  $55 \pm 10$  years; diabetes duration: nil;  $6 \pm 6$ ;  $38 \pm 11$ ;  $36 \pm 15$  and  $39 \pm 11$  years; and eGFR:  $96 \pm 16$ ;  $107 \pm 21$ ;  $90 \pm 20$ ;  $85 \pm 26$ ; and  $62 \pm 29$  ml/min/1.73m<sup>2</sup>; ( $p < 0.001$  for all). Mean 24-hour CASP was  $114 \pm 17$ ;  $115 \pm 13$ ;  $121 \pm 13$ ;  $119 \pm 16$  and  $122 \pm 14$  mmHg; ( $p < 0.001$ ). Mean 24-hour systolic BP (SBP) was  $122 \pm 19$ ;  $124 \pm 15$ ;  $129 \pm 15$ ;  $128 \pm 17$  and  $132 \pm 15$  mmHg; ( $p < 0.001$ ). Central dipping was  $10.0 \pm 5.9$ ;  $11.1 \pm 7.9$ ;  $8.7 \pm 6.1$ ;  $8.9 \pm 6.6$  and  $6.6 \pm 6.6$ %; ( $p < 0.001$ ). Brachial dipping was  $10.6 \pm 5.2$ ;  $11.2 \pm 6.4$ ;  $9.5 \pm 5.3$ ;  $9.4 \pm 5.6$  and  $7.5 \pm 5.9$ %; ( $p < 0.001$ ). Correlations between BPs and dipping were  $r^2 = 0.98$  and  $r^2 = 0.93$  ( $p < 0.001$  and  $p < 0.001$ ). Following multivariate adjustment (age, gender, 24-hour mean arterial pressure, 24-hour heart rate, BMI) both CASP and SBP increased with diabetes duration and albuminuria ( $p < 0.001$ ). Systolic and central dipping decreased with duration and albuminuria ( $p = 0.012$  and  $p < 0.013$ ).

**Conclusion:** 24-hour CASP and brachial BP measurements were feasible in patients with type 1 diabetes. 24-hour CASP and brachial BPs increased, while brachial and less so central dipping decreased with diabetes duration and albuminuria, independently of covariates.

Clinical Trial Registration Number: NCT01171248



## 1245

**Carotid intima-media thickness is reduced 12 months after gastric bypass surgery in patients with type 2 diabetes**L. Lundby-Christensen<sup>1</sup>, L. Tarnow<sup>1</sup>, A. Vaag<sup>2</sup>, N. Wiinberg<sup>3</sup>, D. Hansen<sup>4</sup>, D. Worm<sup>4</sup>, L. Hvolris<sup>4</sup>, L. Naver<sup>4</sup>, T. Almdal<sup>1</sup>;<sup>1</sup>Steno Diabetes Center, Gentofte, <sup>2</sup>Rigshospitalet, Copenhagen,<sup>3</sup>Frederiksberg Hospital, <sup>4</sup>Hvidovre University Hospital, Denmark.

**Background and aim:** Patients with type 2 diabetes (T2D) have increased risk of cardiovascular disease (CVD). Whether normalised glycaemic control reduces this risk remains still unknown. Laparoscopic Roux-en-Y gastric bypass (RYGB) has been shown to improve or even normalise blood glucose in patients with T2D. RYGB is therefore a unique in vivo model to investigate potential changes in risk of CVD that follows improved glycaemic control. Carotid intima-media thickness (carotid IMT) measured by ultrasound is a well established marker of CVD. We aimed to investigate the effects of RYGB on the progression of carotid IMT in severely obese patients with and without T2D.

**Materials and methods:** Thirtyfour patients (hereof 16 with normal glucose tolerance (NGT) and 18 with T2D) were examined before and 6 and 12 months after RYGB. At all examinations mean and maximum carotid IMT of the common carotid artery were measured using B-mode ultrasound (GE logic 9 with a 9 linear (8 MHz) or 12 linear (12 MHz) probe. All dynamic sequences were stored and analysed afterwards using specialised software.

**Results:** All patients performed as expected a significant weightloss 12 months after RYGB (-29.8 kg,  $p < 0.01$  (T2D) and -30.6 kg,  $p < 0.01$  (NGT)). Among the T2D patients who prior to RYGB received glucose lowering drugs (either tablets and/or insulin), 80% of the patients could terminate this treatment and HbA1c decreased despite this with 0.66% ( $p < 0.01$ ). Mean carotid IMT decreased 0.041 mm among patients with T2D 12 months after RYGB ( $p > 0.01$ ) and decreased 0.010 mm among patients with NGT ( $p = 0.44$ ). The change in mean IMT was not significantly different in patients with and without T2D ( $p = 0.11$ ). Maximum carotid IMT decreased 0.070 mm 12 months after RYGB among patients with T2D ( $p > 0.01$ ) and decreased 0.041 mm ( $p = 0.02$ ) among patients with NGT. The change in maximum IMT was not significantly different in patients with and without T2D ( $p = 0.25$ ). Statistical analyses were performed using SAS statistics and the proc mixed procedure.

**Conclusion:** Significant reduction in mean carotid IMT was found in patients with T2D 12 months after RYGB but not among patients without T2D. However significant reduction in maximum carotid IMT was found both in patients with and without T2D 12 months after RYGB. Even though the changes in carotid IMT was more pronounced among patients with T2D, which could be due to the improved glycaemic control, the difference in patients with and without T2D was not significant.

*Supported by: Aase and Einar Danielsen foundation*

## 1246

**Skin autofluorescence in type 1 diabetes mellitus**M. Genevieve<sup>1,2</sup>, C. Gonzalez<sup>3,2</sup>, A. Vivot<sup>4,2</sup>, C. Raffaitin<sup>3,2</sup>, H. Gin<sup>3,2</sup>, V. Rigalleau<sup>3,2</sup>;<sup>1</sup>Nephrologie, Hôpital Pellegrin, Bordeaux, <sup>2</sup>Université Bordeaux 2 Victor Segalen, Bordeaux, <sup>3</sup>Diabétologie-Nutrition, Hôpital Haut-leveque, Pessac,<sup>4</sup>Santé publique, Hôpital Pellegrin, Bordeaux, France.

**Background and aims:** Since skin autofluorescence can assess the subcutaneous accumulation of fluorescent advanced glycation end products, we investigated whether it was linked with glycaemic control and complications of type 1 diabetes mellitus.

**Materials and methods:** Using the AGE Reader™, we measured the forearm skin autofluorescence in 302 patients with type 1 diabetes: 174 men, age: 50 (37–61) years, diabetes duration: 21 (11–31) years, followed up every six months. We examined its association with their glucose control: last HbA1c and mean of the last five HbA1c. The skin autofluorescence values were compared in the patients with and without diabetic complications. The association between the skin autofluorescence and the glucose control were tested by linear regression analysis. The skin autofluorescence from subjects with or without complications were compared by non-parametric tests.  $P < 0.05$  was considered significant.

**Results:** The skin autofluorescence was 2.0 (1.7–2.4) arbitrary units (AU), correlated with age and diabetes duration (respectively  $r = 0.20$  and  $r = 0.15$ ,  $p < 0.0001$ ). After adjustment for age and diabetes duration, it was related to the 2–3 years ( $\beta = 0.13$ ,  $p = 0.0001$ ,  $r = 0.24$ ,  $n = 247$ ). Skin autofluorescence was

higher in the patients with severity of complications (no retinopathy  $n = 88$ , skin autofluorescence 2.2 (1.8–2.5) AU,  $p < 0.001$  versus others), proliferative retinopathy ( $n = 41$ , 2.5 (2–2.7) AU,  $p < 0.05$  versus background), macroalbuminuria ( $n = 10$ , 3.0 (2.5–3.9) AU,  $p < 0.001$  versus others), and macroangiopathy ( $n = 24$  previous cardiovascular event, 2.4 (2.0–2.7) AU,  $p < 0.005$  versus others). 3/5 patients had higher than theoretical skin autofluorescence, although their last HbA1c and diabetes duration were similar to the others, they had 2 to 3 times more severe microangiopathic complications.

**Conclusion:** Skin autofluorescence is related to long-term glucose control and diabetic complications.

## PS 110 Effects of medication on cell culture models

1247

### Inhibitory effect of irbesartan on HMGB1 production in macrophages

Y. Kato, K. Kato, H. Kamiya, A. Watarai, E. Naito, J. Nakamura;  
Division of Diabetes, Department of Internal Medicine, Diabetes Center,  
Aichi Medical University, Nagakute, Japan.

**Background and aims:** High-mobility group box 1 (HMGB1) is released into the extracellular space by activated macrophages and acts as an inflammatory mediator through the receptor for advanced glycation endproducts (RAGE) or toll-like receptor (TLR). Recent studies reported that the number of HMGB1-producing macrophages significantly increased in human atherosclerotic lesions and the serum HMGB1 concentrations were significantly increased in type 1 and type 2 diabetic patients, suggesting that HMGB1 plays an important role in the development of diabetic complications through inflammatory processes. Sub-study of Irbesartan in Patients with type 2 diabetes and Microalbuminuria (IRMA 2) demonstrated that irbesartan treatment reduced biomarkers of inflammatory activities in type 2 diabetic patients. However, the effects of irbesartan on HMGB1 production are still unknown. In the present study, we investigated the effects of irbesartan on HMGB1 production and the molecular mechanisms of its effects in macrophages to evaluate the anti-inflammatory effects of irbesartan.

**Materials and methods:** The murine macrophage cell line, RAW264.7 cells were maintained in RPMI 1640 medium containing 5% heat-inactivated fetal calf serum. RAW264.7 cells were stimulated by lipopolysaccharide (LPS) from *Escherichia coli* O55:B5. HMGB1 or interferon- $\beta$  (IFN- $\beta$ ) was measured by ELISA. Nitric oxide (NO) was measured as its end product, nitrite, using Griess reagent. The cell surface expression of TLR4 was examined by a flow cytometric analysis.

**Results:** Irbesartan inhibited LPS-induced HMGB1 production in RAW264.7 cells ( $101.9 \pm 9.6$  vs.  $36.9 \pm 8.1$  ng/mL,  $p < 0.005$ ). Irbesartan reduced LPS-induced IFN- $\beta$  production ( $162.7 \pm 0.7$  vs.  $58.4 \pm 1.3$  pg/mL,  $p < 0.01$ ). Irbesartan inhibited LPS-induced NO production ( $18.3 \pm 2.1$  vs.  $9.5 \pm 0.4$   $\mu$ M,  $p < 0.01$ ). Losartan did not inhibit LPS-induced HMGB1 production. Irbesartan did not affect the cell surface expression of TLR4.

**Conclusion:** We examined the effects of irbesartan on LPS-induced production of HMGB1, IFN- $\beta$ , and NO in macrophages. Previous studies indicated that LPS exploited TLR4/IFN- $\beta$  signaling pathway to induce HMGB1 release, and that NO played a role as a downstream molecule of IFN- $\beta$  signalling pathway. Irbesartan inhibited HMGB1 production via the inhibitory effect on IFN- $\beta$  signalling pathway in macrophages. Our data indicates that irbesartan would have beneficial effects on diabetic complications through its anti-inflammatory effects.

1248

### Disturbed mitochondrial function can contribute to the metabolic side effects of olanzapine

A. Kolonics, Z. Literáti-Nagy, A. Hidasi, K. Tóty;  
Molecular Pharmacology, N-Gen Research Laboratories, Budapest,  
Hungary.

**Background and aims:** Second generation antipsychotics drugs commonly cause metabolic side effects including rapid development of insulin resistance. Antipsychotic drugs are active on several receptors and activation of hypothalamic AMPK through the histamine H1 receptor has been identified as a key mechanism of the orexigenic effect of antipsychotic drugs. Olanzapine has also a rapid effect on lipid metabolism resulting in inhibition of lipolysis and a switch in fuel preference. Mitochondrial abnormalities are causally linked to certain forms of diabetes, obesity and mental disorders. We analyzed the effects of olanzapine on mitochondrial function to clarify its involvement in pathogenesis of metabolic side effects.

**Materials and methods:** Direct effect of olanzapine on mitochondrial function was evaluated in mitoplast isolated from murine brain and heart. Complex I, II activity and succinate induced reverse electron flow was measured by Amplex Red in functionally intact mitochondria. The effects on mitochondrial ROS production in L6 muscle cells was revealed by MitoSox staining. In vivo effects of olanzapine were studied in mice after single 20 mg/kg i.p. and 10-day 3 mg/kg i.p. treatment. Olanzapine provoked insulin resistance was

studied in healthy individuals by hyperinsulinemic euglycemic clamp method and mitochondrial function in peripheral lymphocytes was evaluated by FACS analysis simultaneously. Molecular interaction between olanzapine and complex I was studied in silico by Vina docking program on complex I structure (Thermus Thermophilus).

**Results:** Daily 10 mg olanzapine resulted in a significant decrease of whole body insulin sensitivity ( $-32 \pm 5\%$ ) in healthy individuals for the 18th day. Analysis of lymphocytes revealed slight increase of mitochondrial mass (MitoTracker staining) and robust increase of ROS production (DCFDA:  $85 \pm 20\%$ ). In brain and heart derived mitoplast olanzapine directly inhibited complex I activity ( $50 \pm 2\%$ ) at high concentration (200–500  $\mu$ M). At therapeutically relevant dose-range (0.3–5  $\mu$ M) olanzapine elevated mitochondrial ROS production in L6 myocytes. In case of 1 h treatment slight increase of basal ROS production was detected in mitoplast isolated from mouse brain and muscle after 1h treatment. Succinate induced ROS production was elevated only in brain mitoplast, with an robust increase of complex I activity. Chronic 10 days olanzapine treatment resulted in weak inhibition of complex I and slight elevation of complex II activity in both types of tissues. Molecular docking analysis revealed binding of olanzapine to NADH binding site of Nqo1 subunit with comparable binding energy to NADH (dG= -8.6 kcal/mol).

**Conclusion:** Data indicate that olanzapine rapidly induced insulin resistance in healthy individuals that was associated with a change in mitochondrial function of peripheral lymphocytes. In silico docking revealed a potential binding of olanzapine on complex I. Olanzapine induced disturbance of mitochondrial function, the elevated ROS production a modulation of complex I activity may contribute to the metabolic side effects of the drug.

Supported by: OTKA NN 76716

1249

### High glucose effects on eNOS and SIRT1 expression and chromatin acetylation in endothelial cells

A. De Marco<sup>1,2</sup>, G. Formoso<sup>1,2</sup>, N. Di Pietro<sup>3,2</sup>, A. Pandolfi<sup>3,2</sup>, V. De Laurenzi<sup>3,2</sup>, M. Federici<sup>4</sup>, A. Consoli<sup>1,2</sup>;

<sup>1</sup>Department of Medicine and Aging, University of Chieti, <sup>2</sup>G. d'Annunzio University Foundation (CeSI), Chieti, <sup>3</sup>Department of Biomedical Sciences, University of Chieti, <sup>4</sup>Department of Internal Medicine, University of Rome Tor Vergata, Italy.

**Background and aims:** The “bad metabolic legacy” exposing to increased vascular risk diabetic individuals who experienced poor glycemic control in the early stages of the disease might be mediated by hyperglycemia related epigenetic modifications in the vascular cells. SIRT1 is an important regulator of metabolism and stress response. As an NAD<sup>+</sup>-dependent protein deacetylase, SIRT1 regulates gene expression programs in response to cellular metabolic status, modulating chromatin function via several epigenetic mechanisms such as direct deacetylation of histones, recruitment of other nuclear enzymes to chromatin or deacetylation of transcription factors and coregulators. Since increased eNOS expression and activity have been described among the hyperglycemia related endothelial cells modifications, we set to investigate eNOS, SIRT1 and chromatin epigenetic modifications (Histones H3 Lysine acetylation) following high glucose exposure in endothelial cells.

**Materials and methods:** HUVEC and HAEC were cultured in Normal Glucose (NG, 5 mmol/L) and High Glucose (HG, 25 mmol/L) conditions for 48 hours. eNOS and SIRT1 protein levels were assessed by Western Blot analysis. In order to evaluate the role of SIRT1 in modulating eNOS expression, HUVEC and HAEC were transfected by electroporation (Amaza<sup>®</sup> Nucleofector<sup>®</sup> Technology, LONZA) to over-express (SIRT1+) or down-regulate (SIRT1-) SIRT1 expression. Chromatin Immunoprecipitation was performed on transfected HAEC to analyze the acetylation levels of Histone H3 at eNOS promoter. Data are expressed as fold increase over NG condition ( $\pm$  Standard Deviation). Statistical analysis were performed using the One-Way ANOVA or Student *t*-test. Value of  $p < 0.05$  were considered statistically different.

**Results:** At the Western Blot analysis, as compared to the respective control cells, eNOS and SIRT1 protein levels were found to be increased by 30% ( $\pm 0.18$ ) and 38% ( $\pm 0.07$ ) respectively in HUVEC exposed to HG, and by 68% ( $\pm 0.038$ ) and 40% ( $\pm 0.13$ ) respectively in HAEC exposed to HG. In order to verify the effects of SIRT1 on eNOS expression, HUVEC and HAEC were transfected to over-express (SIRT1+) or down-regulate (SIRT1-) the expression of SIRT1. In HUVEC SIRT1+, eNOS resulted increased by 66% ( $\pm 0.3$ ) when cultured in NG and by 52% ( $\pm 0.21$ ) when cultured in HG. This increase was even larger in HAEC SIRT1, where it was of 2.6 folds when cells were cultured in HG and of 2.3 folds when cultured in NG. We performed Chromatin

Immunoprecipitation (ChIP) on transfected HAEC exposed to NG and HG for 48 hours to analyze Histone H3 acetylation levels at the eNOS promoter. As compared to NG HAEC, H3 acetylation level resulted higher ( $29\% \pm 0.17$ ) in the proximal promoter region (-166/-26 bp) in HAEC exposed to HG. In HAEC SIRT1+, H3 acetylation levels at distal promoter and proximal promoter regions were  $60\% (\pm 0.049)$  and  $97\% (\pm 0.07)$  greater than in control HAEC when both cultured in NG.

**Conclusion:** Taken together, these data suggest a possible role for SIRT1 in modulating the expression of eNOS in endothelial cells in response to metabolic stress like hyperglycemia and that such modulation could, at least in part, be mediated by epigenetic mechanisms.

*Supported by: EFSD/Sanofi grant*

## 1250

### The vascular protective effect of GLP-1 on HMVEC in hyperglycaemic conditions

D.-M. Lim<sup>1</sup>, K.-Y. Park<sup>1</sup>, B.-J. Kim<sup>1</sup>, J.-Y. Kim<sup>2</sup>;

<sup>1</sup>Endocrinology and Metabolism, Konyang university Hospital, Daejeon,

<sup>2</sup>Soonchunhyang Medical Science Research Institute, School of Medicine, Soonchunhyang University, Chunan, Republic of Korea.

**Background and aims:** The major complication of diabetes mellitus is cardiovascular problem. But the aspects of vascular complication are some different in macrovascular and microvascular features. So, this study was conducted to investigate the different response and mechanism of human microvascular endothelial cell (HMVEC) and human aortic endothelial cell (HAEC) in high glucose and in pretreatment with exendin-4 (Ex-4), a GLP-1 receptor agonist.

**Materials and methods:** Expression of the Notch-1, 4 and GLP-1 receptor was examined in HMVEC and HAEC by RT-PCR and western blot analysis. The different response of HMVEC and HAEC in high glucose (30 mM) after pretreatment with Ex-4 (25 nM) was evaluated based on real-time PCR, western blot analysis, immunocytochemistry, wound healing assay and angiogenesis assay. Also, we investigate the association with the different response of GLP-1 and the signaling of Notch-1 in HMVEC and HAEC using Notch-1 siRNA.

**Results:** GLP-1 receptor, notch 1 and 4 were expressed in HMVEC and HAEC. Although, there was no angiogenesis and wound healing effect in high glucose stressed HAEC and/or pretreatment with VEGF or Ex-4, but in HMVEC, angiogenesis and wound healing effect were shown. Also, Notch-1 signaling was changed in HMVEC. MMP-2, PECAM-1 and TIMP-2 were increase in HMVEC with pretreatment Ex-4. To investigate whether the angiogenesis effects of Ex-4 are related Notch-1, the effect of down-regulation Notch was examined using a siRNA method in HMVEC. Branch number of angiogenesis, wound healing effect and MMP-2 were significantly decreased in Notch-1 siRNA treated HMVEC.

**Conclusion:** Our findings suggested that regulation of Notch-1 signaling in Ex-4 treated HMVEC induce angiogenesis and wound healing effect. Modulation of Notch 1 signaling may hold promise as a novel therapeutic strategy for the treatment of diabetic microvascular complications.

## 1251

### Exendin-4 protected human umbilical vein endothelial cells from tunicamycin-induced apoptosis via inhibiting IRE1 $\alpha$ /JNK/caspase-3 pathway

L. Wu, L. Liu;

Department of Endocrinology, Fujian Institute of Endocrinology, Union Hospital of Fujian Medical University, Fuzhou, China.

**Background and aims:** The survival of endothelial cells is important to maintain the integrality of vascular structure and function. The unusual increase of apoptosis of endothelial cells is a significant factor for atherosclerosis. The goal of this study was to explore the effect of Exendin-4, a glucagon-like peptide-1 receptor agonist, on tunicamycin-induced apoptosis in human umbilical vein endothelial cells (HUVECs).

**Materials and methods:** All studies were performed in primary HUVECs treated with tunicamycin with or without Exendin-4 pretreatment. Markers of cell viability and apoptosis were assessed in all cells, as well as the protein expression levels of IRE1 $\alpha$ , p-IRE1 $\alpha$ , JNK, p-JNK and caspase-3.

**Results:** Following tunicamycin administration, HUVECs viability was gradually reduced in a dose- and time-dependent manner, and fluorescence microscopy confirmed that tunicamycin was inducing HUVEC apoptosis. This

apoptotic effect was attenuated by Exendin-4 pretreatment. The cell viability gradually increased with pretreatment of Exendin-4 (5–150 nmol/L), compared with tunicamycin-alone group. Fluorescence microscopic analysis with Hoechst/PI staining showed that the apoptotic rate of HUVECs decreased 40% in Exendin-4-pretreatment group than that of tunicamycin-alone group ( $P < 0.01$ ). Similarly, the ratio of p-IRE1 $\alpha$ /IRE1 $\alpha$ , p-JNK/JNK and active caspase-3/procaspase-3 were increased by tunicamycin (10  $\mu$ g/ml), an effect that was counteracted by Exendin-4. Pretreatment of HUVECs with exendin-4 resulted in a 23.25% decrease of the expression of p-IRE1 $\alpha$  than that of tunicamycin-alone group, and resulted in 29.25% and 22.2% decreases of p-JNK and active caspase-3, respectively ( $P < 0.01$ ). The effect of exendin-4 was similar to that of tauroursodeoxycholic acid (TUDCA). Compared with tunicamycin-alone group, the ratios of p-IRE1 $\alpha$ /IRE1 $\alpha$ , p-JNK/JNK and active caspase-3 /procaspase-3 were all reduced both in exendin-4-pretreatment group and TUDCA-pretreatment group.

**Conclusion:** This study demonstrates that Exendin-4 can protect HUVECs from tunicamycin-induced apoptosis. Furthermore, our data suggests that the mechanism for this effect is mediated by inhibiting the IRE1 $\alpha$ /JNK/caspase-3 pathway.

## 1252

### Metformin protects endothelial cells from methylglyoxal-mediated damage

E.J. Pilavachi, N. Murphy, J. Mabley;

School of Pharmacy and Biomolecular Sciences, University of Brighton, UK.

**Background and aims:** Methylglyoxal (MGO), a glycolysis derived reactive dicarbonyl compound has been implicated as a central mediator of diabetic complications in both type I and type II diabetes. MGO has already been shown to cause endothelial cell dysfunction via increased oxidative stress. Aminoguanidine is a known scavenger of MGO and can protect endothelial cell function from MGO-mediated damage. Metformin has a similar structure to aminoguanidine and has also been shown to be capable of scavenging MGO though not as effectively. The aim of this study is to investigate whether metformin is able to protect endothelial cell function from MGO-mediated dysfunction.

**Materials and methods:** The effects of MGO on acetylcholine-mediated NO dependent vasorelaxation were measured using ex vivo rat aortic rings exposed to 0.3 mM MGO for 4h, metformin (0.3 and 0.5 mM) was co-administered to investigate any protective effect. In vitro experiments using the EA.Hy926 human endothelial cell line were performed examining the effect of MGO  $\pm$  metformin on cell viability (MTT assay), necrosis and apoptosis levels (propidium iodide/Hoechst staining followed by morphological analysis) and cellular oxidative stress (NBT assay).

**Results:** MGO (0.3 mM) exposure increased the IC<sub>50</sub> for acetylcholine-mediated relaxation from  $0.06 \pm 0.01$   $\mu$ M to  $0.315 \pm 0.1$   $\mu$ M ( $P < 0.05$ ), with metformin at 0.25 and 0.5 mM reducing this to  $0.09 \pm 0.02$   $\mu$ M and  $0.06 \pm 0.01$   $\mu$ M respectively ( $p < 0.05$  vs. MGO alone). Metformin also protected the endothelial cell line from MGO-mediated loss of cell viability significantly increasing it from  $65 \pm 3\%$  with MGO 0.6 mM alone to  $90 \pm 2\%$  with 0.5 mM metformin ( $p < 0.05$  vs. MGO alone). MGO 0.3 mM increased both necrosis (from  $6 \pm 2\%$  to  $15 \pm 4\%$  ( $p < 0.05$ )) and apoptosis (from  $1.3 \pm 0.7\%$  to  $3.3 \pm 0.8\%$  ( $p < 0.05$ )) levels in endothelial cells. Metformin at 0.25 mM and 0.5 mM was again able to significantly protect against the MGO-mediated increase in cell death reducing necrosis to  $6.3 \pm 3\%$  and  $5 \pm 2\%$  and apoptosis to  $2.1 \pm 0.5$  and  $1.8 \pm 0.2$  respectively. Metformin was also able to reduce the levels of cellular oxidative stress observed following MGO exposure.

**Conclusion:** Metformin was able to provide significant protection against MGO-mediated endothelial cell dysfunction. Metformin also protected the endothelial cell line from MGO-mediated decrease in cell viability and increase in necrosis and apoptosis. This protective effect may be mediated by metformin scavenging MGO and hence preventing the increased cellular oxidative stress. These data suggest that metformin, apart from its glucose-lowering effect, may also provide direct protection to the cardiovascular system in diabetes.



## 1253

### Effect of sulfonylurea, glinide and insulinotropic imidazoline compounds on endothelial cell survival

S.V. Zaitsev, I.I. Zaitseva, P.-O. Berggren;

The Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska Institutet, Stockholm, Sweden.

**Background and aims:** Hyperglycaemia induces dysfunction and damage of vascular endothelial cells leading to diabetic complications. The aim of this study was to investigate the effects of insulinotropic compounds (RX871024, efaroxan, glibenclamide, repaglinide) and elevated glucose on endothelial cells in the absence or presence of vascular endothelial growth factor (VEGF), which under normal conditions enhances endothelial cell proliferation, survival and nitric oxide (NO) production.

**Materials and methods:** Human umbilical vein endothelial cells (HUVEC) were cultured with the compounds at a high glucose concentration in the presence or absence of VEGF and viability, proliferation and NO production were measured. Cell viability was detected by using MTS tetrazolium compound after 9 days culture. Assessment of HUVEC proliferation was performed by measuring BrdU incorporation into the DNA of dividing cells for 90 h. NO production was measured as  $\text{NO}_2^-$  release into the medium by using Griess reagent after 40 h incubation.

**Results:** After culture at 25 mM glucose HUVEC viability was diminished to 50% ( $p<0.05$ ) when compared with a normal glucose concentration of 5.5 mM. High glucose decreased HUVEC proliferation to 85% ( $p<0.05$ ) compared to normal glucose. Presence of 100 ng/ml VEGF in culture media elevated HUVEC viability at 5.5 mM glucose, but had no effect on high glucose induced reduction in HUVEC viability. Despite addition of VEGF, 25 mM glucose still reduced HUVEC proliferation to 85% compared to 5.5 mM glucose and VEGF ( $p<0.05$ ). Reduction of HUVEC viability and proliferation were in line with a decrease in NO production induced by elevated glucose both in the presence or absence of VEGF. Exposure HUVEC to 50  $\mu\text{M}$  RX871024 at 25 mM glucose in the presence or absence of VEGF drastically decreased endothelial cell viability, to a level of about 10% of that at 25 mM glucose without the imidazoline ( $p<0.005$ ). In contrast, 50  $\mu\text{M}$  efaroxan had no effect on endothelial cell viability in the absence of VEGF, however, decreasing HUVEC viability in the presence of VEGF to 60% ( $p<0.005$ ). Both in the absence and in the presence of VEGF RX871024 reduced HUVEC proliferation to a level of about 50% of that at 25 mM glucose without the imidazoline ( $p<0.005$ ). In comparison efaroxan did not influence HUVEC proliferation regardless the presence of VEGF. Neither of the imidazoline compounds affected significantly HUVEC NO production at elevated glucose concentration regardless the presence of VEGF. Exposure of endothelial cells to 2  $\mu\text{M}$  glibenclamide or 2  $\mu\text{M}$  repaglinide at elevated glucose concentration in the absence of VEGF reduced HUVEC viability to a level of about 70% of that at 25 mM glucose ( $p<0.05$ ). Addition of VEGF abrogated the ability of glibenclamide and repaglinide to diminish HUVEC viability at high glucose concentration. Neither glibenclamide nor repaglinide affected HUVEC NO production in the absence of VEGF. Repaglinide, but not glibenclamide, in the presence of VEGF elevated HUVEC NO production by 80% ( $p<0.05$ ). Neither glibenclamide nor repaglinide affected HUVEC proliferation regardless the presence of VEGF.

**Conclusion:** Our data demonstrate an important interplay between the insulinotropic compounds, the VEGF and the ambient glucose concentration affecting the survival of the vascular endothelial cells. Consequently, this interplay needs to be taken into consideration when designing novel oral insulinotropic compounds for the treatment of diabetes.

*Supported by: Swedish Foundation for Strategic Research, Novo Nordisk Foundation*

## 1254

### Glucagon like peptide-1 protect diabetic cardiomyopathy by inactive endoplasmic reticulum stress pathway

J. Liu, Y. Liu, L. Chen, Y. Wang, X. Song;

The Second Hospital of Jilin University, Changchun, China.

**Background and aims:** Diabetic cardiomyopathy is a heart muscle specific disease which is attributed to abnormal cellular metabolism. This study is aimed to investigate whether endoplasmic reticulum stress (ER stress) is involved in the pathogenesis of diabetic cardiomyopathy and glucagon like peptide-1 (GLP-1) analog liraglutide can protect diabetic cardiomyopathy by inhibiting this pathway.

**Materials and methods:** Type 2 diabetic cardiomyopathy rat models were established by high fat diet for eight weeks and STZ injection 30mg/kg twice. The models are verified by glucose test, echocardiography exam and HE staining for heart tissue. The rats were divided into six groups: non-diabetic cardiomyopathy (CON) group, diabetic cardiomyopathy rats without GLP-1 (DC) treatment, diabetic cardiomyopathy rats with high, medium and low-dose GLP-1 (LIRA-H, LIRA-M, LIRA-L) respectively treatment, and diabetic cardiomyopathy rats with insulin (INS) treatment. The levels of blood glucose, body weight and cardiac function by echocardiography exam were recorded both at baseline and treatment for four weeks and eight weeks. The hemi-quantitative reverse transcription PCR (RT-PCR) were performed to analyze mRNA levels of ATF6, TRAF2 and XBP1, which were considered as the hallmarks genes of ER stress. The expression of ER stress associated proteins including glucose-regulated protein 78 (Grp78), enhancer-binding protein homologous protein (CHOP) and Caspase-12 were also detected by Western blot.

**Results:** The glucose levels are decreased in LIRA or INS group and Liraglutide can improve the cardiac functions which can not been found in INS group ( $p<0.05$ ). The expressions of ATF4, TRAF2 and XBP1 are significantly increased in DC group compared with CON group ( $p<0.05$ ). And GLP-1 treatment can decrease the expression of these genes. The levels of Grp78, CHOP and Caspase-12 also up-regulated in DC group but liraglutide can inactive the expressions of these ER stress related genes ( $p<0.05$ ).

**Conclusion:** Liraglutide can protect diabetic cardiomyopathy by inactive ER stress pathway. The improvement of cardiac function by liraglutide are independent of glucose control.

*Supported by: NSFC(NO30971398), the technology Development Grant of Jilin Province*

## PS 111 Macrovascular complications in experimental studies

### 1255

#### Glyoxalase-I overexpression partially prevents diabetes-induced impaired arteriogenesis in rats

O. Brouwers<sup>1</sup>, L. Yu<sup>1</sup>, P. Niessen<sup>1</sup>, J. Slenter<sup>1</sup>, A. Wagenaar<sup>1</sup>, T. Miyata<sup>2</sup>, M. Post<sup>1</sup>, W. Backes<sup>1</sup>, C. Stehouwer<sup>1</sup>, M. Huijberts<sup>1</sup>, C. Schalkwijk<sup>1</sup>;

<sup>1</sup>Maastricht University Medical Centre, Netherlands, <sup>2</sup>Tohoku University, Sendai, Japan.

**Background and aims:** Arteriogenesis is an important mechanism in improving the outcome of ischemic vascular diseases. However in patients who suffer from diabetes, this adaptive collateral capacity is impaired. Hyperglycemia is the initial factor of diabetes induced vascular damage, but the mechanisms involved are poorly understood. The advanced glycation endproduct (AGE) precursor methylglyoxal (MGO) is a highly reactive oxo-aldehyde, which can be detoxified by the enzyme glyoxalase-I. We hypothesized that diabetes causes dysregulation of the GLO-I enzyme, and that this dysregulation is required for reduced collateral artery remodeling capacity. Therefore we used a GLO-I overexpressing transgenic diabetic rat model with a unilateral femoral artery ligation, to investigate the role of MGO in diabetes-induced impaired arteriogenesis.

**Materials and methods:** Wild-type and GLO-I transgenic rats which were non-diabetic or diabetic (65mg/kg streptozotocin) for a period of 12 weeks were subjected to ligation of the right femoral artery. Blood flow recovery after ligation of the femoral artery was measured by laser Doppler perfusion imaging (LDPI) immediately, 3 and 6 days after ligation. Formation of collateral arteries was measured by in vivo time-of-flight magnetic resonance angiography (MRA) at 7-Tesla before euthanization on day 7.

**Results:** Diabetes resulted in significantly ( $p<0.05$ ) decreased GLO-I protein and activity levels in hind limb skeletal muscle ( $5.1\pm0.3$  U/mg) compared with wild type control rats ( $6.2\pm0.4$  U/mg). GLO-I activity in the skeletal muscle of the transgenic control ( $62.2\pm1.7$  U/mg) and transgenic diabetic group ( $63.3\pm4.2$  U/mg) was approximately 10 times higher than in the wild-type animals. LDPI analyses showed a significantly ( $p<0.05$ ) decreased blood perfusion recovery after 6 days in the diabetic animals ( $45.7\pm0.7$  %) compared with control animals ( $64.5\pm1.0$ ), without any beneficial effect of GLO-I overexpression in the diabetic animals ( $46.7\pm0.9$ %) nor in the control GLO-I animals ( $64.1\pm2.8$  %). MRA analyses showed a significant ( $p<0.05$ ) decrease in the number of collaterals in the wild type diabetic animals (median 1.0, range 0-3) compared with the control animals (median 6.0, range 4-9). GLO-I overexpression partially prevented this decrease in collateral numbers (median 3.0, range 1-5),  $p<0.05$  in the diabetic animals. However GLO-I overexpression did not have any effect in the non-diabetic state (median 5.5, range 3-9).

**Conclusion:** Defective GLO-I might contribute to diabetic impairment of arteriogenic adaptation to ischemia, since the impairment can be rescued by overexpressing the enzyme. In diabetic models with presumed microvasculopathy, laser Doppler imaging may not be suitable to measure the functional improvement by arteriogenesis.

### 1256

#### The tissue damage in cardiac ischaemia-reperfusion injury is reduced under the fed state cellular energy metabolism

M. Dambrova<sup>1,2</sup>, J. Kuka<sup>1</sup>, M. Makrecka<sup>1,2</sup>, E. Liepinsh<sup>1</sup>;

<sup>1</sup>Latvian Institute of Organic Synthesis, <sup>2</sup>Riga Stradins University, Riga, Latvia.

**Background and aims:** It has been hypothesized that better survival of ischemic myocardium cells can be achieved by stimulating glucose oxidation, either directly or secondarily, to partially inhibited FA oxidation. Healthy subjects, depending on the availability of glucose or fatty acids (FA), are able to switch appropriately between the available energy substrates. At a postprandial state, the glucose and insulin concentrations are increased, and glucose oxidation is facilitated. In contrast, in a fasted state, the glucose concentration is significantly lower, and energy metabolism is switched to FA. The aim of the present study was to evaluate the ischemic damage in the heart tissues under fed and fasted states in healthy and diabetic animals with respect to the balance in glucose and fatty acid metabolism.

**Materials and methods:** In fed and fasted Wistar and diabetic Goto-Kakizaki (GK) rats, the blood plasma biochemical parameters were measured. The myocardial infarction experiment in isolated hearts from fed and fasted rats was performed according to the Langendorff technique. The infarct size was calculated as percentage of necrotic tissue in the risk area. In addition, the rate of glucose and FA oxidation and the expression of genes related to energy metabolism were assessed in isolated hearts from fed and fasted rats. All data are expressed as the mean  $\pm$  S.E.M. For statistical analyses, the one-way ANOVA followed by Newman-Keuls Multiple Comparison Test or Student's *t*-test were used.

**Results:** Under the fed state the Wistar rat heart tissues oxidized about 10-fold more glucose and 3-fold less palmitate than under the fasted state ( $p<0.05$ ). The shift in favoured energy substrate was reflected by changes in the insulin and PPAR- $\alpha$ /PGC-1 signalling pathway-regulated expression of genes related to glucose and FA metabolism, respectively. In isolated Wistar rat heart infarction experiment the shift towards glucose metabolism was accompanied by the statistically significant 2.7-fold reduction in the infarct size under fed state ( $27\pm6$ %) as compared to fasted state ( $73\pm5$ %). In GK rats, the infarct size was also reduced in fed ( $46\pm12$ %) in comparison to fasted ( $89\pm4$ %) rat hearts ( $p<0.05$ ). However, the overall infarct sizes in GK rat hearts were increased as compared to Wistar rat hearts which might be related to the insulin resistance-limited oxidation of glucose in GK rat tissues.

**Conclusion:** Our results demonstrate that the cardiac recovery from ischemia-reperfusion injury is more efficient under the fed state due to the facilitated glucose metabolism. Meanwhile, the ischemic injury is more severe under the fasted state due to the prevailing FA metabolism. The switch from FA to glucose oxidation is critical for the survival of cardiac tissues after ischemia-reperfusion reperfusion and long-term fasting might provoke more severe cardiac events in diabetic patients.

Supported by: Latvian State Research Program BIOMEDICINE

### 1257

#### Differences in the development of atherosclerosis between two lines of selectively bred mice with different susceptibilities to diet-induced glucose intolerance

A. Asai, M. Nagao, M. Kawahara, Y. Sato, Y. Nakajima, H. Sugihara, S. Oikawa;

Endocrinology and Metabolism, Nippon Medical School, Tokyo, Japan.

**Background and aims:** Epidemiological studies indicate that postprandial hyperglycaemia is an independent risk factor for atherosclerotic cardiovascular disease. However, there have been few suitable animal models for understanding the involvement of blood glucose fluctuations in the pathogenesis of atherosclerosis in vivo. We recently established two lines of mice with different susceptibilities (prone and resistant) to high fat diet (HFD)-induced glucose intolerance by selective breeding (designated SDG-P and SDG-R, respectively). After receiving HFD (32% energy as fat) for 5 weeks, SDG-P mice show evident glucose intolerance in OGTT relative to SDG-R. In this study, we assessed differences in the development of atherosclerosis in the newly established two lines of mice with different glucose intolerance.

**Materials and methods:** Female mice of each strain (SDG-P and SDG-R) fed an atherogenic diet (AD; 1.25% w/w cholesterol, 36% energy as fat) for 20 weeks (8-28 weeks of age). OGTT (2.0 g/kg body weight) was performed before and after the AD feeding. For quantitative analysis of atherosclerotic lesion formation, serial frozen sections (10  $\mu$ m thickness, spanned 450  $\mu$ m) of aortic sinus were prepared after the 20-week AD feeding. The lesion size was calculated from Oil Red O-stained area (mean of 9 sections, each separated by 50  $\mu$ m). Data are expressed as mean $\pm$ SD. Differences between strains were assessed using Student's *t* test. Correlations between lesion area and blood measurements were examined by Pearson's correlation analysis.

**Results:** In OGTT, SDG-P mice showed modest glucose intolerance as compared with SDG-R mice even before AD feeding (SDG-P vs SDG-R: AUC<sub>0-120 min</sub>;  $1424\pm289$  vs  $1148\pm170$  mmol/l $\cdot$ min,  $n=8-9$ ,  $p=0.035$ ). The differences in glucose tolerance between the two strains became more evident after receiving AD for 20 weeks (AUC<sub>0-120 min</sub>;  $1605\pm446$  vs  $738\pm113$  mmol/l $\cdot$ min,  $n=9$ ,  $p<0.0001$ ). After AD feeding, mean aortic sinus lesion area in SDG-P mice was approximately 3-fold greater than that in SDG-R mice ( $12.9\pm7.6$  vs  $4.2\pm3.4 \times 10^3 \mu\text{m}^2$ ,  $n=12-14$ ,  $p=0.0012$ ). No significant differences were observed in plasma cholesterol levels (total, HDL, nor non-HDL) between the two strains, whereas SDG-P mice showed higher plasma triacylglycerol concentration than SDG-R mice ( $p=0.0052$ ). Aortic sinus lesion area was strongly correlated with blood glucose levels in OGTT (AUC<sub>0-120 min</sub>;  $r=0.67$ ,

$p=0.0023$ ), whereas no significant correlations were observed between the lesion area and any of the plasma lipid measurements.

**Conclusion:** These results demonstrate that HFD-induced glucose intolerance-prone mice (SDG-P) are more susceptible to atherosclerotic lesion formation relative to glucose intolerance-resistant mice (SDG-R). The newly established two lines of mice with different glucose intolerance may be useful animal models for investigating the involvement of blood glucose fluctuations in the pathogenesis, prevention, and treatment of atherosclerotic complications.

## 1258

### Natural products to treat diabetes: effects of acute and chronic administration of aloe vera in a mouse model of type 2 diabetes

Y. Brito-Casillas<sup>1</sup>, J.C. Wiebe<sup>1</sup>, L. López-Ríos<sup>1</sup>, C. Muñoz-Mediavilla<sup>2</sup>, A. Arín-Martínez<sup>1</sup>, J. Nóvoa<sup>1,3</sup>, A.M. Wäagner<sup>1,3</sup>;

<sup>1</sup>Servicio de Endocrinología, Unidad de Investigación del Complejo Universitario Insular Materno Infantil de Gran Canaria, Las Palmas de Gran Canaria, <sup>2</sup>Universidad de Cádiz, <sup>3</sup>Departamento de Ciencias Médicas y Quirúrgicas, Universidad de Las Palmas de Gran Canaria, Spain.

**Background and aims:** Natural products are a potential source of new treatments for diabetes. Aloe vera has shown glucose-lowering effects in human and animal studies, but more accurate evaluations are required. Our aims were to evaluate acute and chronic effects of aloe administration in diabetic C57BL/6 mice.

**Materials and methods:** 16 weeks old C57BL/6 ( $n=10/\text{group}$ , 50% males) were fed a 60%-fat diet for at least 20 weeks. In order to assess its short-term effects, several doses of aloe leaf pulp extract (11–20% acemannan) or saline was administered orally with 2 g glucose/Kg during a glucose tolerance test (OGTT), on different days, in a randomised cross-over design. The most effective dose was fed in the diet for 90 days to diabetic C57BL/6 mice and compared with high-fat diet only ( $n=10/\text{group}$ , 50% males). Body weight (BW), food and water consumption, welfare state and external appearance were assessed weekly.  $\text{HbA}_{1c}$  (DCA, Siemens), OGTT and intra-peritoneal insulin tolerance tests (ITT) were performed before and after treatment. Glucose (Glucocard G+) and insulin (ELISA) concentrations were measured. AUCs were calculated by the trapezoid rule. Cold allodynia response was assessed by the acetone test, where higher scores indicate more severe neuropathy. Blood sampling and macroscopic organ evaluation, were performed post-mortem. For comparisons between groups, Wilcoxon's test or Student's test for paired data were used. A two-tailed  $p<0.05$  was considered significant.

**Results:** Short-term experiments showed a hypoglycaemic effect of aloe, when compared to saline (at 15 min  $240.1 \pm 25.3$  vs  $311.9 \pm 54.9$  mg/dl,  $p=0.003$ ; AUC  $270.8 \pm 34.6$  vs  $307.3 \pm 61.7$ ,  $p=0.016$ ). After chronic treatment, no significant differences were observed between groups overall, regarding OGTT, ITT,  $\text{HbA}_{1c}$  or the rest of blood tests. Response to cold allodynia, however, was improved in the treatment group ( $2.08 \pm 0.51$  vs  $2.63 \pm 0.23$ ,  $p=0.006$ ) at the expense of the female mice. In the treated female mice, final  $\text{HbA}_{1c}$  was lower than in controls ( $4.76 \pm 0.17$  vs  $4.84 \pm 0.35$ ,  $p=0.049$ ), whereas glucose concentrations during the ITT (at 15 min  $100.7 \pm 14$  vs  $75.9 \pm 14$  mg/dl;  $p=0.024$ ) and insulinemia during the OGTT (at 60 min,  $0.41 \pm 0.03$  vs  $0.1 \pm 0.09$  ng/ml,  $p=0.044$ ) were higher. Treated males showed a higher final BW ( $54.9 \pm 2.1$  vs  $50.7 \pm 3.4$ ,  $p=0.045$ ), a relatively higher weight of the pancreas (pancreas/BW) ( $0.56 \pm 0.003$  vs  $0.48 \pm 0.004$  %,  $p=0.012$ ) and a relatively lower left paragenital fat weight ( $0.76 \pm 0.14$  vs  $1.4 \pm 0.17$  %,  $p=0.001$ ). A similar, non-significant trend was found in females. In addition, potential signs of ageing, such as grey hair and alopecia appeared an average of 35 days later in the treatment than in the control group.

**Conclusion:** Orally administered aloe vera acutely reduces blood glucose in diabetic mice. Longer-term treatment increases insulin secretion and improves glucose control in females. Cold allodynia inhibition could be a result of better glucose control, but a direct effect of aloe on pain or on neuropathy itself cannot be ruled out. External appearance, pancreas and fat changes will be further evaluated.

## 1259

### Superoxide anion overproduction, impaired eNOS and enhanced adhesion molecule expression in human endothelial cells exposed to maternal diabetes

S.A.A. Sultan, A.M. Graham;

Medical Sciences, University of Bradford, UK.

**Background and aims:** Epidemiological studies suggest a link between foetal exposure to maternal diabetes and propensity to develop various diseases including cardiovascular disease in adulthood. The present study measured levels of superoxide anion production and determined effects on expression of endothelial nitric oxide synthase (eNOS) and adhesion molecules in HUVEC from diabetic mothers compared to healthy mothers (control) under normoglycemic and hyperglycemic conditions.

**Material and methods:** Intracellular superoxide was measured by both nitrotriazolium blue (NBT) and 2',7'-Dichlorodihydrofluorescein Diacetate (DCFH-DA) experiments. Expression of eNOS mRNA was quantified by real time PCR. The expression of adhesion molecules E-selectin and intracellular adhesion molecule-1 (ICAM-1) were determined by immunofluorescence staining.

**Results:** Both NBT and DCFH-DA experiments showed that diabetic HUVEC cultured in physiologic glucose concentrations for 1–3 days generated excess ROS ( $p<0.05$ ) compared to control cells. However, exposure of diabetic HUVEC to elevated glucose (29mM) had no additional effect. Expression of eNOS mRNA was significantly reduced in diabetic HUVEC compared to control at 5 mM glucose ( $p<0.001$ ). A similar reduction was observed in diabetic HUVEC incubated at 29mM glucose for 10 days when compared to same cells at 5mM glucose ( $p<0.05$ ). We also report for the first time the increased expression of E-selectin ( $p<0.05$ ) and ICAM-1 ( $p<0.05$ ) in unstimulated diabetic HUVEC compared to control.

**Conclusion:** This study showed that exposure of HUVEC to maternal diabetes causes irreversible alterations in endothelial cell gene expression and function in cultured cells even after glucose normalisation and may contribute to increased risk of cardiovascular diseases in adulthood.

Supported by: Saudi Arabian Cultural Bureau, London

## 1260

### Role of p66Shc in the modulation of endothelial leukocyte recruitment

M.R. Orlando, L. Laviola, M. Incalza, A. Leonardini, A. Cignarelli, F. Tortosa, R. Labarbuta, S. Perrini, A. Natalicchio, F. Giorgino; Endocrinology & Metabolic Diseases, University of Bari, Italy.

**Background and aims:** Endothelial cells actively participate in inflammatory events leading to atherogenesis by regulating leukocyte recruitment via the expression of adhesion molecules. The protein p66Shc has been proposed as a sensor/generator of oxidative stress and a mediator of vascular dysfunction. The aim of this work was to investigate the role of p66Shc in cytokine-triggered leukocyte recruitment by human umbilical vein endothelial cells (HUVEC).

**Materials and methods:** Expression and phosphorylation levels of specific signaling molecules were assessed by immunoblotting techniques. Intracellular ROS generation, in the presence of the DHE probe, was evaluated by fluorimetric analysis. Gene expression was evaluated by real-time RT-PCR. Wild-type p66Shc and mutant p66Shc, in which Ser36 was replaced by Ala (p66Shc-Ala36), were selectively overexpressed in HUVEC by infecting cells with recombinant adenoviruses. Selective silencing of p66Shc was obtained with specific siRNAs. Leukocyte transmigration was detected with a commercially available kit.

**Results:** Exposure of HUVEC to 50 ng/ml TNF- $\alpha$  resulted in increased phosphorylation of p66Shc on Ser36 and intracellular ROS levels, and promoted leukocyte transmigration through the HUVEC monolayer ( $p<0.05$ ). TNF- $\alpha$  also induced a marked increase in the expression of the adhesion molecule E-Selectin ( $p<0.05$ ). Adenovirus-mediated overexpression of p66Shc in HUVEC resulted in enhanced p66Shc phosphorylation on Ser36, increased ROS and E-Selectin levels, and marked augmentation of leukocyte transmigration ( $p<0.05$ ), which appeared to be further increased when cells were exposed to TNF- $\alpha$ . Conversely, overexpression of a phosphorylation-defective p66Shc protein, with Ser36 mutated to Ala, did not augment ROS levels or E-Selectin expression beyond those found in wild-type cells, and modestly increased leukocyte transmigration, with an additional effect of TNF- $\alpha$  exposure comparable to that found in control cells. Moreo-



ver, silencing of p66Shc with two independent siRNA sequences resulted in marked reduction of E-Selectin expression, both under basal conditions and following TNF- $\alpha$  exposure ( $p < 0.05$ ), which was associated with decreased leukocyte transmigration ( $p < 0.05$ ).

**Conclusion:** The p66Shc protein acts as a novel intermediate in the TNF- $\alpha$  signaling pathway regulating the adhesive properties of endothelial cells, which involve at least in part changes in E-Selectin expression. The effects of p66Shc in this pathway require cytokine-mediated phosphorylation of the protein on Ser36.

## 1261

### Impaired pancreatic blood flow due to high-fat diet (HFD); pancreatic fluoro-2-deoxy-D-glucose ( $[^{18}\text{F}]\text{FDG}$ ) kinetics and validation

H. Honka<sup>1</sup>, J.C. Hannukainen<sup>2</sup>, M. Tarkia<sup>2</sup>, V. Oikonen<sup>2</sup>, A. Saraste<sup>2</sup>, M. Teräs<sup>2</sup>, J. Knuuti<sup>2</sup>, V. Fagerholm<sup>1</sup>, K. Mikkola<sup>1</sup>, P. Nuutila<sup>2</sup>;

<sup>1</sup>University of Turku, <sup>2</sup>Turku PET Centre, Finland.

**Background and aims:** Mechanisms behind  $\beta$ -cell exhaustion and ensuing hyperglycaemia in obesity include lipotoxicity, endoplasmic reticulum (ER) and oxidative stress, and systemic inflammation. Furthermore, pancreatic microvascular dysfunction and subsequent islet ischaemia has been recently hypothesized to trigger vicious cycle ultimately leading to  $\beta$ -cell insufficiency. Positron emission tomography with computed tomography (PET-CT) is a sophisticated method of studying parameters in organs with compromised accessibility. Aim of the present study was to validate the use of PET for pancreas and to investigate the effects of high-fat diet on pancreatic perfusion and glucose uptake.

**Materials and methods:** Eighteen pigs were randomized into an intervention group and control group. Pigs in the intervention group received first streptozotocin injections and thereafter high-fat diet (HFD) for 6 months. Diet containing either 15 % lard + 1.5 % cholesterol ( $n = 6$ ) or 20 % lard + 4 % cholesterol ( $n = 4$ ). The control group received regular farm nutrition. Fasting state PET-CT scans with sequential radiowater ( $[^{15}\text{O}]\text{H}_2\text{O}$ ) and  $[^{18}\text{F}]\text{FDG}$  boluses were performed and blood was sampled to measure plasma biochemical and metabolic parameters. After the scanning, sacrifice and laparotomy was done, and pancreas was explanted to measure autoradiographic accumulation of  $^{18}\text{F}$ , and *ex vivo* tissue radioactivity. Pancreatic volumes-of-interest (VOIs) were drawn to head, corpus, and tail of the pancreas. Glucose uptake was measured from *ex vivo* tissue samples, and with fractional uptake rate (FUR) and Gjedde-Patlak plot. Blood flow was calculated using one-tissue compartmental model.

**Results:** High fat diet was associated with weight gain ( $127 \pm 7.6$  vs.  $83 \pm 9.2$  kg,  $p < 0.001$ ), hypercholesterolaemia ( $12 \pm 3.5$  vs.  $2.1 \pm 0.3$  mmol/l,  $p < 0.001$ ), and hyperglycaemia ( $12 \pm 2.2$  vs.  $6.0 \pm 2.2$  mmol/l;  $p < 0.01$ ). Blood flow in the pancreas was lower in the intervention group ( $35 \pm 16$  vs.  $57 \pm 26$  ml/100g $\cdot$ min;  $p < 0.05$ ), but baseline glucose uptake rates were similar between groups ( $0.082 \pm 0.054$  vs.  $0.063 \pm 0.027$   $\mu\text{mol}/\text{ml}\cdot\text{min}$ , NS). PET derived  $[^{18}\text{F}]\text{FDG}$  influx values were correlated with values measured from *ex vivo* tissue samples ( $r = 0.63$ ,  $p < 0.01$ , and  $r = 0.87$ ,  $p < 0.01$  for FUR and Gjedde-Patlak to *ex vivo*, respectively). Pancreatic glucose uptake was homogeneous in both groups with no identifiable islets.

**Conclusion:** The results of our study indicate that PET-CT is feasible for the assessment of pancreatic metabolism and perfusion. Secondly, perfusion is blunted after long lasting HFD intervention. Since islets are normally highly perfused and consume nearly 20 % of pancreatic total blood flow, this dysfunction might cause subtle islet ischaemia and  $\beta$ -cell insufficiency in time.

Clinical Trial Registration Number: ESAVI-2010-03970/Ym-23

Supported by: Sigrid Juselius

## 1262

### In vitro determination of the hepatotoxic effects of methylglyoxal

T. Loganathan<sup>1</sup>, T. Buxton<sup>1</sup>, J. Mabley<sup>2</sup>;

<sup>1</sup>Brighton and Sussex Medical School, <sup>2</sup>School of Pharmacy & Biomolecular Sciences, University of Brighton, UK.

**Background and aims:** Methylglyoxal (MGO), a glycolysis derived reactive dicarbonyl compound has been implicated as a central mediator of diabetic complications. Recently diabetes has been found to be an independent risk factor for development of liver disease including non-alcoholic fatty liver disease (NAFLD). Hepatocyte exposure to saturated fatty acids results in loss of cell viability, increase in apoptosis and increased inflammation as dem-

onstrated by IL-8 release leading to NFLD, as diabetes increases the risk of developing this disease we hypothesize that MGO may have similar effects on hepatocytes as fatty acids. The aim of this study therefore was to determine the effects of MGO on hepatocytes.

**Materials and methods:** The human hepatocyte cell line, HepG2, was exposed to MGO (0.1–1 mM) for 24 h. Cell viability was determined using the MTT assay and cell death assessed by propidium iodide/Hoechst staining. Oxidative stress was measured using the nitroblue tetrazolium assay. Inflammation was assessed by IL-8 release measured using a commercially available ELISA kit. HepG2 cells were also exposed to MGO (0.6–1 mM)  $\pm$  aminoguanidine (0.1–0.5 mM) and again cell viability, death, oxidative stress and IL-8 was measured.

**Results:** Hepatocytes exposed to MGO for 24 h showed a dose-dependent decrease in cell viability with 0.6 mM and 0.8 mM reducing it to  $68 \pm 2\%$  and  $54 \pm 3\%$  respectively ( $p < 0.05$  vs. untreated cells). There was also an increase in both necrosis, from  $7 \pm 2\%$  to  $42 \pm 2\%$  and  $48 \pm 1\%$ , and apoptosis, from  $0.5 \pm 0.3\%$  to  $3.4 \pm 1$  and  $2.8 \pm 0.5\%$ , for 0.6 mM and 0.8 mM MGO respectively ( $p < 0.05$  vs. untreated cells). Both 0.6 mM and 0.8 mM MGO also increased oxidative stress following a 6 h exposure by  $40 \pm 2\%$  and  $60 \pm 5\%$  respectively ( $p < 0.05$  vs. untreated cells). IL-8 release was increased by MGO concentrations as low as 0.3 mM with a  $20 \pm 7\%$ ,  $29 \pm 1\%$  and  $38 \pm 2\%$  increase observed with 0.3, 0.6 and 0.8 mM respectively ( $p < 0.05$  vs. untreated cells). All these effects were reversed by simultaneous addition of aminoguanidine (0.5 mM) with cell viability being returned to  $86 \pm 2\%$  and  $77 \pm 3\%$  for 0.6 and 0.8 mM MGO ( $p < 0.05$  vs. MGO alone) and similar protective effects observed on cellular oxidative stress, necrosis and apoptosis levels and IL-8 release.

**Conclusion:** Physiological concentrations of MGO reduced hepatocyte cell viability and increased levels of both necrosis and apoptosis. In addition inflammation as assessed by hepatocyte release of IL-8 was also increased by MGO. These effects may be mediated by the increased cellular oxidative stress observed in hepatocytes following MGO exposure. Aminoguanidine, a scavenger of MGO, protected hepatocytes from the MGO-mediated damaging effects. In conclusion, MGO has similar effects on hepatocytes as fatty acids suggesting a possible synergistic effect between fatty acids and MGO that may explain why diabetics have an increased risk of developing liver diseases such as NAFLD.

## 1263

### Intramuscular allogeneic mesenchymal stem cells are of comparable efficacy to syngeneic MSCs in hind limb ischaemia of diabetic animals

A. Liew<sup>1</sup>, C. Baustian<sup>1</sup>, D. Thomas<sup>1</sup>, E. Vaughan<sup>1</sup>, X. Chen<sup>1</sup>, C. Sanz Nogues<sup>1</sup>, M. Creane<sup>1</sup>, S. Alagesan<sup>1</sup>, P. Owens<sup>2</sup>, J. Horan<sup>1</sup>, R. Ceredig<sup>1</sup>, P. Dockery<sup>3</sup>, M. Griffin<sup>1</sup>, A. Duffy<sup>4</sup>, T. O'Brien<sup>1</sup>;

<sup>1</sup>Regenerative Medicine Institute, <sup>2</sup>Centre for Microscopy and Imaging and NBIPI, <sup>3</sup>Department of Anatomy, <sup>4</sup>Medtronic, Galway, Ireland.

**Background and aims:** Peripheral arterial disease (PAD) is prevalent and is associated with an increased risk of death and ischemic events. PAD is associated with a worse outcome in the presence of diabetes mellitus. Mesenchymal stem cells (MSCs) have been demonstrated to enhance therapeutic neovascularisation. Allogeneic MSCs have an advantage in that they may be used as 'off the shelf' product as they can be prepared in advance and they can be isolated from non-diabetic donors thus avoiding the problem related to sufficient cell number and disease-induced cellular dysfunction. The direct comparison of the efficacy of transplantation of allogeneic and syngeneic MSC transplantation from diabetic and non-diabetic donors respectively into a diabetic animal model is currently unknown.

**Materials and methods:** Syngeneic and allogeneic MSCs were isolated from diabetic obese dbdb/C57BKS(H-2d) and non-diabetic C57BL/6(H-2b) mice respectively. Angiogenesis array was performed on the MSC conditioned media. Normal saline, allogeneic or syngeneic MSCs ( $1 \times 10^6$ ) were administered intramuscularly into the ischaemic thigh of diabetic obese dbdb/C57BKS(H-2d) following the induction of unilateral hindlimb ischaemia (HLI). Blood perfusion and clinical assessment of the ischaemic limbs were determined twenty one days following the induction of HLI.

**Results:** MSCs derived from both sources secrete various angiogenesis related factors. Some of these factors were found at a lower amount or absent in the conditioned media of the dbdb/C57BKD(H-2d) group as compared to the C57BL/6(H-2b) group. Syngeneic MSCs significantly improved blood perfusion as compared to the control group only at week two. However, allogeneic MSCs significantly improve blood perfusion as compared to the control group at all time points studied after transplantation. The improvement of blood perfusion in both the allogeneic and syngeneic MSC groups was not

statistically different. The percentage of toe necrosis were 86%(6/7), 86%(6/7) and 50%(4/8) in the control, syngeneic and allogeneic groups respectively. Toe amputation was only seen in the control group (14%) but not in the other groups.

**Conclusion:** Both autologous or allogeneic MSC transplantation have similar efficacy in the diabetic hindlimb ischaemic setting.

*Supported by: Medtronic and Science Foundation Ireland*

## PS 112 Diabetes and sleep apnoea

### 1264

#### Prevalence of obstructive sleep apnoea in type 2 diabetes patients

L. Tarnow<sup>1</sup>, H. Storgaard<sup>2</sup>, T.P. Almdal<sup>2</sup>, M. Laub<sup>3</sup>;

<sup>1</sup>Clinical Research Unit, Steno Diabetes Center, <sup>2</sup>Steno Diabetes Center, Gentofte, <sup>3</sup>Respiratory Centre East, Rigshospitalet, Copenhagen, Denmark.

**Background and aims:** Obstructive Sleep Apnoea (OSA) is associated with increased cardiovascular mortality and morbidity. We, therefore, aimed to investigate the prevalence of OSA among type 2 diabetes (T2DM) patients referred to a tertiary referral centre.

**Materials and methods:** All patients referred to the T2DM clinic during 10 months were offered to participate in a three-stage screening program for the diagnosis of OSA; 1. The Berlin questionnaire. A “high likelihood of OSA”-score qualified for; 2. an outpatient ApneaLink® monitoring. Patients with a positive test (apnea-hypopnea index (AHI) > 5/hour) were referred to; 3. diagnostic polygraphy (with Embletta®) in a sleep clinic.

**Results:** A total of 180 patients (men, 61%; age  $59.7 \pm 10.5$  years, BMI,  $31.8 \pm 6.7$  kg/m<sup>2</sup>, diabetes duration  $8.2 \pm 6.3$  years, HbA1c,  $7.3 \pm 1.2\%$ ) participated. Amongst these 104 (61%) had a “high likelihood of OSA”-score according to the Berlin questionnaire, and 77 patients (43%) were after ApneaLink® study referred to the sleep clinic. So far, polygraphy has been performed in 49 patients, and 44 have been diagnosed with OSA - corresponding to an estimated prevalence of OSA of 34 % of the entire study population (n=180). ApneaLink® positive (n = 77) patients had higher BMI ( $34.3 \pm 7.4$  versus  $30.3 \pm 5.7$  kg/m<sup>2</sup>,  $p < 0.001$ ), higher HbA1c ( $7.6 \pm 1.3$  versus  $7.2 \pm 1.1\%$ ,  $p = 0.008$ ) and lower HDL-cholesterol ( $1.1 \pm 0.3$  versus  $1.3 \pm 0.4$  mmol / l,  $p = 0.003$ ) as compared with those without symptoms (n = 77) or signs of OSA on ApneaLink® (n = 26). The groups were comparable with respect to: blood pressure, albuminuria, p-creatinine, retinopathy, LDL cholesterol, treatment with oral antidiabetic drugs, RAS blockade and statins. In a multiple linear regression analysis, AHI increased significantly with higher age, increased BMI and declining HDL-cholesterol, whereas sex, HbA1c and insulin dose per kg bodyweight were not related to AHI.

**Conclusion:** Every third T2DM patient referred for treatment in a hospital outpatient clinic have OSA. Increased focus on screening, diagnosis and treatment of OSA in T2DM patients is needed.

### 1265

#### Prevalence and clinical characteristics of insulin dependent patients suffering from sleep apnoea syndrome

A. Iosup, J. Courrèges, H. Banciu, J. Thuan, N. Vigier Simorre, H. Bonneure; Centre Hospitalier Général de Narbonne, France.

**Background and aims:** If the frequency of the sleep apnea syndrome (SAS) is noticeable in the general population and a major issue for type 2 diabetic patients (T2D), its prevalence and characteristics have not really been studied in type 1 diabetic patients. Thus the following research aims at determining SAS' prevalence and its characteristics in type 1 diabetic (T1D) patients.

**Materials and methods:** 81 T1D patients over 18 years old (average age  $53.4 \pm 14.6$  years, sex ratio M/F 1.04, diagnosed with diabetes for  $23.3 \pm 12.9$  years) were enrolled in chronological order. They benefited from a systematic SAS screening on a clinical level (Epsworth scale + simplified Berlin scale) and a respiratory level (pulse oxymetry). In case of abnormality and/or doubt, we performed a polysomnography (EPS, n=36) in order to confirm SAS diagnosis. Patients diagnosed with SAS were classified into 2 groups: severe SAS or moderate SAS. Severe SAS was defined by apnea hypopnea index (AHI)  $\geq 30$ /h and moderate SAS by AHI [10;30] with a number of micro awakenings  $\geq 10$ /h.

**Results:** 22 (27.1%) of T1D patients were diagnosed with obstructive SAS (average AHI:  $26.8 \pm 11.8$  /h), among which 12 (14.8%) were classified as severe SAS (average AHI:  $37.3 \pm 7.7$  /h) and 10 (12.3%) as moderate SAS (average AHI:  $18 \pm 5.8$  /h + average number of micro awakenings:  $142 \pm 103$ /night). We compared clinical and oxymetry characteristics of patients suffering from SAS (n=22) with those of patients with no SAS (n=59). At least 2 out of 4 clinical signs were significantly noticed (90 vs 50% -  $p < 0.001$ ) in SAS patients (snoring: 65 vs 27%, discontinuous sleep: 50 vs 23%, morning asthenia : 55 vs 19 % and day somnolency : 55 vs 19 %), as well as oxymetry results (SAS vs no SAS): desaturation index  $10.6$  vs  $1.6$  -  $p < 0.0001$  and lowest SpO<sub>2</sub>: 76 vs 84 % -  $p < 0.004$ . There were no differences (SAS vs no SAS) in diabetes

duration (24.9 vs 22 years), HbA1c (7.8 vs 8.1%), blood pressure (124/76 vs 126/72 mmHg), BMI (25.4 vs 24.5 kg/m<sup>2</sup>) and lipid status. There were differences (SAS vs no SAS) in age ( $62 \pm 13$  vs  $49 \pm 14$  years -  $p < 0.01$ ), percentage of males (72.7 vs 45.8 % -  $p < 0.03$ ), macroangiopathy frequency (76.2 vs 35.2 % -  $p < 0.001$ ) and microangiopathy frequency (95.5 vs 61.5 % -  $p < 0.003$ ), and particularly autonomic neuropathy (and not peripheral neuropathy) with 71.4 vs 21 % -  $p < 0.004$  (mostly unfelt hypoglycemia 63.6 vs 22.6 % -  $p < 0.0007$ ).

**Conclusion:** SAS frequency although poorly explored in T1D patients, is significantly noticeable (27%). The clinical profile of T1D SAS patients is very different from the one of general population or of T2D patients. This study identifies in elderly, normal weighted patients, an increase in macro and microangiopathy (especially autonomic neuropathy) frequency, therefore creating new physio pathological research axes.

## 1266

### A new device for screening severe form of obstructive sleep apnoea in patients with type 2 diabetes

J.-P. Le Berre<sup>1</sup>, G. Chaumet<sup>2</sup>, A. Courson<sup>1</sup>, H. Moussa Ali<sup>3</sup>, B. Colle<sup>1</sup>, C. Mounier<sup>1</sup>, O. Coste<sup>1</sup>;

<sup>1</sup>Department of endocrinology, Hospital Desgenettes, Lyon, France, <sup>2</sup>UMR MD2, University of Marseille, France, <sup>3</sup>Military Health Service of Djibouti, Republic of Djibouti.

**Background and aims:** Obstructive sleep apnoea syndrome (OSAS) is a highly prevalent comorbidity of type 2 diabetes. Patients with type 2 diabetes have high risk of cardiovascular disease and OSAS is often misestimated. The aim of this study was to evaluate the ability of an automatic respiratory events recorder, i.e. the RU SLEEPING (PHILIPS-RESPIRONICS inc), in severe OSAS detection. In this study, the results obtained with this device were systematically compared with the index determined by polysomnography in patients with type 2 diabetes.

**Materials and methods:** We performed a prospective study, including 64 patients with type 2 diabetes. Two nights sleep recordings staggered-in-time were realised for each patient: the first one with RU SLEEPING system and the second one with polysomnography (PSG). Two criteria were obtained from RU SLEEPING system: the mean Respiratory Event Index (REI) and the maximum Respiratory Event value (max-REV) observed per hour of recording. Then, both criteria were compared to the gold standard, i.e. the Apnea-Hypopnea Index (AHI) determined by PSG using a linear correlation method. The performances of the RU SLEEPING in the detection of OSAS (AHI > 10) and severe form of OSAS (AHI > 30) were then studied using ROC curves and best kappa method for the determination of cut-off values. Contingency tables were built and sensitivity (Se), specificity (Sp) and positive and negative predictive values (PPV and NPV) were finally calculated from these tables.

**Results:** According to method of linear regression, m-REI and max/h REI were correlated with AHI ( $r = 0.71$  and  $r = 0.69$ ). Area under curve (AUC) of ROC curve was to 0.84 (when AHI > 10) and to 0.86 (when AHI > 30) for REI. AUC was to 0.87 (when IHA  $\geq 10$  and  $\geq 30$ ) for max-REV. According to the best kappa method, the values of cut-off were respectively: for IAH > 10, REI = 3 RE/h (Se = 97 %, Sp = 33%, PPV = 93%, NPV = 50%) and max-REV = 15 RE (Se = 93 %, Sp = 50%, PPV = 95%, NPV = 43%) and for IAH > 30, REI = 12 RE/h (Se = 78 %, Sp = 84%, PPV = 83%, NPV = 79%) and max-REV = 43 RE (Se = 81 %, Sp = 78%, PPV = 79%, NPV = 81%).

**Conclusion:** These results showed that RU SLEEPING system may constitute a valuable device for screening severe forms of obstructive sleep apnea syndrome in patients with type 2 diabetes, compared to polysomnography. The values of best cut-off were 12 respiratory events per hour for REI and 43 respiratory events for max-REV.

Supported by: Philips-Respironics inc.

## 1267

### Blood glucose improvement ameliorates nocturnal arterial oxygen saturation in type 2 diabetic patients: a case-control study

A. Lecube<sup>1</sup>, A. Ciudin<sup>1</sup>, G. Sampol<sup>2</sup>, S. Valladares<sup>1</sup>, J. Mesa<sup>1</sup>, C. Hernández<sup>1</sup>, R. Simó<sup>1</sup>;

<sup>1</sup>Endocrinology Department, Diabetes and Metabolism Research Unit., CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Instituto de Salud Carlos III, Institut de Recerca i Hospital Universitari Vall d'Hebron,

<sup>2</sup>Sleep Unit, Pneumology Department., CIBER Enfermedades Respiratorias. Instituto de Salud Carlos III, Institut de Recerca i Hospital Universitari Vall d'Hebron, Barcelona, Spain.

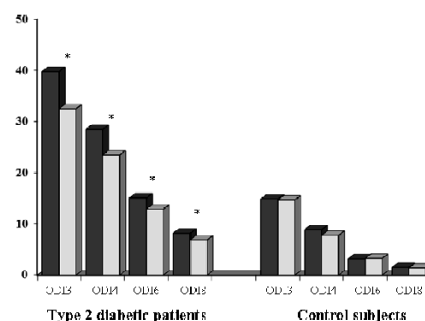
**Background and aims:** There is growing evidence suggesting a deleterious effect of type 2 diabetes on lung function and sleep breathing, and diabetes has been described as an independent risk factor for severe nocturnal hypoxemia. Our aim was to determine the effect of blood glucose control improvement on nocturnal arterial oxygen saturation.

**Materials and methods:** A case-control study including 30 type 2 diabetic patients and 10 non-diabetic subjects, closely matched by age, gender, and BMI, was conducted. The nocturnal oxygen desaturation index (ODI) was calculated at baseline and 5-day after blood glucose control had improved. Four different oxygen desaturation thresholds (reductions in SaO<sub>2</sub>  $\geq 3\%$ ,  $4\%$ ,  $6\%$ , and  $8\%$ ) as indicators of hypoxemia severity (ODI-3%, ODI-4%, ODI-6%, and ODI-8%) were used.

**Results:** At baseline, diabetic patients showed a significantly higher percentage of ODI-3%, ODI-4%, and ODI-6% in comparison with non-diabetic subjects. Fasting plasma glucose and HbA1c positively correlated with the cumulative percentage of time spent with oxygen saturation below 90% (CT90). In addition, multiple linear regression analyses showed that HbA1c was independently associated with ODI-3%, ODI-4%, and ODI-6%. A significant reduction in ODI-3% ( $39.8 \pm 27.5$  vs.  $32.5 \pm 27.5$  events/hour,  $p < 0.001$ ), ODI-4% ( $28.5 \pm 23.7$  vs.  $23.5 \pm 24.2$ ,  $p < 0.001$ ), and ODI-6% ( $15.1 \pm 16.8$  vs.  $12.9 \pm 17.3$ ,  $p = 0.009$ ) was observed after 5 days of blood glucose optimization. By contrast, no changes in ODI events were observed in non-diabetic patients after 5-days of follow-up. Finally, no significant changes in weight were observed in both groups.

**Conclusion:** Our baseline results confirm and extend previous reports concerning the impairment of nocturnal oxymetry that occurs in diabetic patients. Glycemic control improvement reduces the increase of nocturnal ODI that exists in T2DM. The reason why the improvement of glycemic control results in less nocturnal hypoxia remains to be elucidated. However, the rapid effect without changes in body weight suggests a central mechanism on respiratory center output.

**Figure.** Changes in oxygen desaturation index per hour (ODI) at baseline (black bars) and at discharge (white bars) in type 2 diabetic patients (after 5-days of glucose improvement) and control subjects.



\*:  $p < 0.05$  between baseline and discharge.

Supported by: CIBERDEM, ISCIII



## 1268

## High prevalence of GAD65 antibodies in type 2 diabetes mellitus females with sleep apnoea syndrome

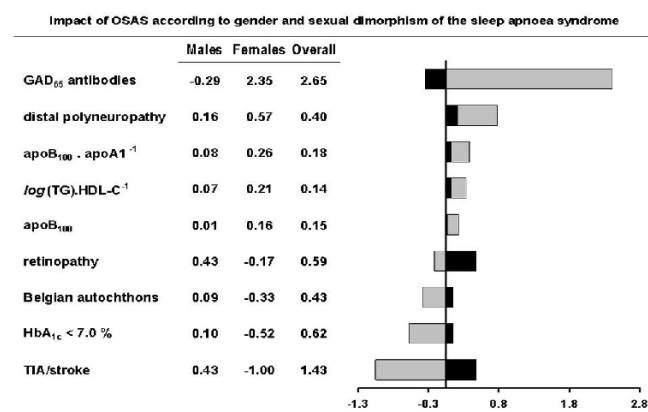
P.B. Katchunga<sup>1</sup>, Y. Mahadeb<sup>2</sup>, S.A. Ahn<sup>3</sup>, M.F. Rousseau<sup>3</sup>, M.P. Hermans<sup>2</sup>, J.C. Cikomola<sup>1</sup>;<sup>1</sup>Université Catholique de Bukavu, Democratic Republic of the Congo,<sup>2</sup>Endocrinology & Nutrition, <sup>3</sup>Cardiology, Université Catholique de Louvain, Brussels, Belgium.

**Background and aims:** Obstructive sleep apnoea syndrome (OSAS) is a frequently overlooked comorbidity in type 2 diabetes (T2DM). OSAS is associated with obesity, metabolic syndrome (MetS), and central fat accretion. OSAS is more frequent in males, who as a result, more often benefit from screening. The study aimed at documenting the barely-assessed sexual dimorphism of OSAS in T2DM beyond common gender differences.

**Materials and methods:** cross-sectional study of 815 adults with T2DM (541 males; 274 females) classified into OSAS[−] and OSAS[+] according to polysomnography's apnoea-hypopnea index, and assessed for cardiometabolic risk factors, glucose homeostasis (HOMA B and S), micro/macrovacular complications, UKPDS 10-year CV risk, thyroid function and autoimmunity (TSH; antiTG, antiTPO; prevalence of autoimmune thyroid disease (AITD); and GAD65 autoantibodies).

**Results:** Overall OSAS prevalence was 16% in the entire group: 18% in males and 11% in females (p 0.0032). There was no significant OSAS-related gender dimorphism for age; education; and diabetes duration. There was a significant dimorphism in ethno-geographical extraction (higher proportion of Maghrebian females with OSAS). There were no sexual-specific dimorphisms related to OSAS in body composition and overall anthropometrics, nor in hypertension prevalence, MetS, HOMA insulin sensitivity [S], HOMA hyperbolic product [BxS] and [BxS] loss rate. There was an OSAS-related gender dimorphism in degree of glucose control, with higher HbA1c (by 7.0 mmol/mol (+12%)) in OSAS [+] females, a mere 17% of whom reaching target (<7.0%) HbA1c (vs. 40% of OSAS [+] males; p 0.0272). There was also an OSAS-related gender dimorphism in apolipoprotein B100 level, higher by 15 mg/dL (+17%) in OSAS [+] females. As regards atherogenic indices, both the apoB100/apoA1 and the log(TG)/HDL-C ratios were sexually-dimorphic in relation to OSAS. Whereas prevalence of AITD was markedly higher in females regardless of OSAS, there was a marked and significant OSAS-related gender dimorphism in frequency of GAD65 antibodies positivity, higher by an absolute 21% (+793%) in OSAS [+] females (vs. OSAS [+] males); and by an absolute 17% (+335%) (vs. OSAS [−] females). There were opposite OSAS-related sexual dimorphisms in microangiopathy, with lesser and higher prevalence of retinopathy and polyneuropathy in females, respectively.

**Conclusion:** We observed significant OSAS-related sexual dimorphisms in GAD65 antibodies, polyneuropathy prevalence, atherogenic dyslipidemia indices [increased in females]; and in retinopathy prevalence, North-Caucasian ancestry, glucose control and TIA/stroke prevalence [lower in females]. These findings raise intriguing questions regarding the reciprocal pathophysiology between obstructive sleep disorders and T2DM.



Numbers represent the relative differences in mean values or proportions between males and females patients with and without OSAS (left columns), and the overall sexual dimorphism in OSAS patients (right column). The graph on the right side represents the male (black bars) and female (grey bars) components of the overall sexual dimorphism of OSAS.

## 1269

## Diabetic retinopathy and sleep apnoea: the Haute-Alsace health network experience

J. Wilhelm<sup>1</sup>, C. Iamandi<sup>2</sup>, A. Derragui<sup>3</sup>, P. Thannberger<sup>3</sup>, S. Moser<sup>1</sup>, P. Guiot<sup>4</sup>, R. Muller<sup>5</sup>, P. Lenoble<sup>6</sup>;

<sup>1</sup>Haute-Alsace Health Network, St Morand Hospital, Altkirch, <sup>2</sup>Department of Functional Explorations, St Morand Hospital, Altkirch, <sup>3</sup>Department of Internal Medicine and Diabetology, Altkirch, <sup>4</sup>AIR à Domicile' association, Mulhouse, <sup>5</sup>Department of Functional Explorations, Altkirch, <sup>6</sup>Department of Ophthalmology - Emile Muller Hospital, Mulhouse, France.

**Introduction:** Sleep apnoea is particularly common in patients with type 2 diabetes, and is not only associated with a higher risk of cardiovascular complications, but also with a higher risk of microangiopathy, especially retinopathy. The effect of treatment with continuous positive airway pressure on the evolution of retinopathy has not been documented to date.

**Materials and methods:** The Haute-Alsace Health Network developed a pluridisciplinary and multi-service platform designed to promote information, prevention, screening, therapeutic education, coordination and support for patients with diabetes. In particular, it includes screening for sleep apnoea and guiding its management. The prevalence of retinopathy was prospectively determined from January 2010, in 114 diabetic patients with sleep apnoea treated with continuous positive airway pressure, and in 641 diabetic patients without known sleep apnoea.

**Results:** In the literature, the prevalence of retinopathy appears to be 50% higher in diabetic patients with sleep apnoea. In the present study, the prevalence of retinopathy in diabetic patients with sleep apnoea treated with continuous positive airway pressure was similar to that of diabetic patients without known sleep apnoea. The effectiveness of noninvasive ventilation increased with the length of the treatment. These results were similar when patients were divided into subgroups according to their hemoglobin A1c levels and the duration of diabetes.

**Conclusion:** In the present study, treatment with continuous positive airway pressure was associated with a reduced risk of retinopathy in diabetic patients with sleep apnoea.

## 1270

## Obstructive sleep apnoea is independently associated with diabetic nephropathy and increased oxidative and nitrosative stress in patients with type 2 diabetes

M. Stevens<sup>1</sup>, A. Ali<sup>2</sup>, S. Begum<sup>3</sup>, K. Dubb<sup>1</sup>, S. Mughal<sup>4</sup>, B. Jose<sup>1</sup>, M.K. Piya<sup>1</sup>, A. Barnett<sup>1</sup>, A.A. Tahrani<sup>1</sup>;

<sup>1</sup>University of Birmingham, <sup>2</sup>University Hospital of Coventry and Warwickshire, Coventry, <sup>3</sup>Birmingham Heartlands Hospital, <sup>4</sup>Birmingham community PCT, UK.

**Background and aims:** Diabetic nephropathy (DN) causes significant morbidity and mortality. Understanding DN pathogenesis is essential to develop new therapies. Obstructive sleep apnoea (OSA) is prevalent in patients with type 2 diabetes (T2D). Since OSA and DN may share common oxidative stress and inflammatory mechanisms, we hypothesized that OSA is associated with DN in patients with T2D.

**Materials and methods:** Adults with T2D were recruited randomly from the diabetes clinic of two UK-based hospitals. Patients with known respiratory disorders (including OSA) and end-stage renal disease were excluded. DN was defined by eGFR of <60 ml/min/1.73m<sup>2</sup> or the presence of albuminuria (2 out of 3 early morning samples) after exclusion of other causes. OSA was assessed using home-based portable multi-channel respiratory device (Alice PDX, Philips Respironics, USA). OSA diagnosed when the apnoea-hypopnea index (AHI) was ≥5 events/hour (OSA+). Serum 3-nitrotyrosine (NT) (nM) and plasma lipid peroxide (LP) (μM/ml) were measured (n=102 samples) by commercial assays as markers of nitrosative and oxidative stress respectively.

**Results:** Two hundred and thirty four patients were included, 64.5% were OSA+ and 39.7% and 36.2% had DN and albuminuria respectively. OSA+ patients were older, more obese and had higher BP compared to those without OSA. DN (29.7% vs. 55.9%, p<0.001) and albuminuria (24.3% vs. 43.2%, p=0.007) prevalence were higher in OSA+ patients. OSA+ patients had lower eGFR (92.9 ± 25.2 vs. 82.4 ± 26.4, p=0.006). OSA+ remained independently associated with DN (OR 2.17, 95%CI 1.03-4.60, p=0.04) after adjusting (using logistic regression) for age, gender, ethnicity, BP, HbA1c, total cholesterol, triglycerides, HDL, diabetes duration, smoking, alcohol, BMI, and the use of insulin, anti-platelet, glucose-lowering, lipid-lowering and anti-hypertensive

treatments. In addition, time spent with oxygen saturation < 80% was an independent predictor of DN (OR 2.85, 95% CI 1.20–6.78,  $p=0.02$ ) and albuminuria (OR 2.74, 95% CI 1.16–6.48,  $p=0.02$ ) after adjustment as in the above mentioned model. OSA+ patients had higher NT (23.53 (16.67–36.07) vs. 15.49 (11.53–24.28),  $p=0.007$ ) and LP (18.39 (8.33–37.40) vs. 7.93 (0.81–22.76),  $p=0.014$ ) levels. NT levels correlated with measures of OSA severity and nocturnal hypoxemia. After adjustment as in the above mentioned model using linear regression, OSA remained independently associated with NT levels ( $p=0.003$ ). AHI ( $B=0.28$ ,  $p<0.001$ ), oxygen desaturation index ODI ( $B=0.27$ ,  $p<0.001$ ), and nadir nocturnal oxygen saturation ( $B=-0.33$ ,  $p=0.006$ ) were also independently associated with NT levels after adjustment. LP correlated significantly with measures of OSA severity and nocturnal hypoxemia severity. After adjustment for age, BMI and diabetes duration LP remained independently associated with ODI ( $r=0.221$ ,  $p=0.031$ ) and nadir nocturnal oxygen saturation ( $r=-0.220$ ,  $p=0.032$ ).

**Conclusion:** We describe a novel association between OSA and DN and nocturnal hypoxemia in patients with T2D and we have identified increased nitrosative and oxidative stress as possible underlying contributors to this association. The ability of OSA treatment to impact DN warrants determination.

*Supported by: NIHR UK and The Novo Nordisk UK Research Foundation*

# Author Index

## A

- Aakhus, S. 1226  
 Aanstoet, H.-J. 963  
 Aas, V. 29  
 Aastrup, T. 442  
 Abate, N. 782  
 Abbas, Z. G. 1170  
 Abbaszadeh, B. 944  
 Abbink, E. J. 929  
 ABCD Nationwide Liraglutide Audit Contributors 801  
 Abdelmoneim, A. 52  
 Abderrahmani, A. 211, 391, 520  
 Abdulkarim, B. 511  
 Åberg Löfvenborg, J. 270  
 Abi Khalil, C. 80  
 Abiko, A. 578  
 Abrahamian, G. 711  
 Abrahamsson, M. 1099  
 Abudukadier, A. 648, 708  
 Accili, D. 141  
 Acerini, C. L. 195  
 Ach, K. 330  
 Achenbach, P. 169  
 Ackermans, M. T. 644  
 Acosta, C. 552  
 Acosta, D. 1061  
 Acquati, S. 1165  
 ACT NOW Investigators 564  
 Adam, K.-P. 108  
 Adams, A. C. 25  
 Adams, J. 245  
 Adams, T. D. 587  
 Adamska, E. 689  
 Adamski, J. 108, 670  
 Adamson, U. 796  
 Ader, M. 439  
 Adhya, S. 1054  
 Admiraal, W. M. 677  
 ADMIRE Study Group 996  
 Afroz, A. 1025  
 Afsana, F. 1232  
 Agarwal, P. K. 851  
 Agarwal, S. 793  
 Agborsangaya, C. B. 350  
 Aggarwal, S. 147  
 Agius, L. 291, 608  
 Agostini, C. 126, 693  
 Agrawal, P. 1059  
 Agudo, J. 444, 447, 448, 633  
 Aguilar, A. 1179  
 Aguilar, S. G. 1179  
 Aguilar-Diosdado, M. 1141  
 Aguilera, E. 164  
 Ah Kioon, M. 428  
 Aharon-Hananel, G. 303, 304  
 Ahlbom, A. 168, 320  
 Ahlqvist, E. 482  
 Ahlström, H. 78  
 Ahmad, A. 1002  
 Ahmann, A. 1042  
 Ahn, S. A. 1268  
 Ahonen, S. 265  
 Åhrén, B. 581, 828, 861  
 Aichler, M. 664  
 Ait Yahia, D. 77  
 Aithal, G. P. 1216  
 Ajdukovic, D. 251  
 Ajjan, R. A. 613  
 Akamizu, T. 686, 1147  
 Akanuma, Y. 319  
 Åkesson, L. 481  
 Akhmetzianova, T. N. 1062  
 Akhrarova, N. 1136  
 Akhyari, P. 92  
 Akimoto, Y. 1124, 1212  
 Akiyama, N. 739  
 Akram, H. 958  
 Al-Hasani, H. 27, 130  
 Al-Oanzi, Z. H. 608  
 Alagesan, S. 1263  
 Alawieh, M. 428, 714  
 Alba, M. 760  
 Albertini, R. 1175  
 Albiero, M. 126  
 Albrecht, T. 67  
 Albus, C. 974  
 Alcarraz-Vizán, G. 540  
 Alegakis, D. 86  
 Alemany, P. 1141  
 Alessi, M.-C. 157  
 Alessi, T. 109  
 Alevizaki, M. 1063, 1080  
 Alexandre, G. D. 987  
 Alexandrou, A. 724  
 Alexiadou, K. 724  
 Alfonso Ross Terres, J. 1028  
 Alhadj Ali, M. 456  
 Ali, A. 1270  
 Ali, L. 681, 1025, 1078  
 Alieva, A. 1136  
 Alikhani, N. 706  
 Alimetova, Z. R. 1062  
 Alkayyali, S. 482  
 Alkemade, G. M. 451  
 Alkhalaf, A. 135, 155  
 Allen, E. 837, 838  
 Allen, J. M. 195  
 Allet, L. 1011, 1190  
 Allgeier, S. 44  
 Alligier, M. 209  
 Almdal, T. P. 253, 370, 377, 1016, 1164, 1245, 1264  
 Almeida, M. 987  
 Almgren, P. 284, 558  
 Almholt, D. L. C. 177  
 Almind, K. 460  
 Alon, T. 118  
 Alpizar, M. 1179  
 Alsalim, W. 828  
 Alt, M. 507  
 Altémir-Trallero, J. 1103  
 Altenhofen, L. 1024  
 Alter, M. 35  
 Altkrüger, A. 398, 410  
 Altomare, M. 115  
 Álvarez, C. 94, 417, 528  
 Alvarez, E. 552  
 Alves, T. 19  
 Alzahrani, S. H. 613  
 Amado, J. 58, 59  
 Amann-Zalan, I. 1036  
 Amati, F. 674, 1011  
 Ambrosioni, E. 1234  
 Ameer, B. 935  
 Amiel, S. A. 359, 378, 381, 441, 728, 958, 1027  
 Amin, F. 1232  
 Amione, C. 137  
 Amisten, S. 381, 385  
 Ammon, H. P. T. 459  
 Amor, A. J. 479  
 Amorese, G. 150  
 Amtoft, M. 377  
 An, S.-Y. 890  
 An, Y. 1204  
 Anastasiou, E. 1063, 1070, 1080  
 Anastasiou-Nana, M. 1240  
 Ancelle, D. 730  
 Ancuta, I. 877  
 Andersen, H. 939  
 Andersen, H. U. 83, 344  
 Andersen, L. 978  
 Andersen, L. W. 46  
 Andersen, M. 594  
 Andersen, M. 626  
 Andersen, M. K. 273  
 Andersen, M. L. M. 238, 275, 276, 614  
 Anderson, J. V. 1140  
 Andersson, T. 168, 317, 320  
 Ando, K. 844, 884, 950  
 Andralojc, K. M. 263  
 Andreozzi, F. 791  
 Andrédóttir, G. 1102, 1146, 1244  
 Anfossi, G. 232  
 Angelopoulos, T. 724  
 Angrisani, L. 586  
 Anguela, X. M. 447  
 Anhalt, H. 1057  
 Anhê, G. F. 652  
 Anichini, R. 702, 1166, 1171  
 Anniballi, G. 878  
 Annuzzi, G. 76, 878  
 Anraku, T. 111  
 Anselmino, M. 583  
 Antenore, A. 343  
 Antonarakis, S. E. 139  
 Antoni, G. 261  
 Antunes, D. D. 554  
 Aoki, K. 318  
 Aouizerat, T. 303, 304  
 AP@home consortium 196  
 Apanovitch, A. M. 721, 743  
 Ara, T. 892  
 Araga, M. 856  
 Aragno, M. 660  
 Arakaki, R. F. 917  
 Araki, A. 319, 1197  
 Araki, E. 661  
 Aranda, G. B. 479  
 Arase, Y. 313, 866  
 Araszkievicz, A. 163  
 Araújo, C. 1096  
 Araujo, R. 419  
 Archibald, L. K. 1170  
 Arden, C. 608  
 Ardestani, A. 522  
 Argento, N. B. 1044  
 Argyrakopoulou, G. 724  
 Arias, P. 605  
 Arín-Martínez, A. 1258  
 Arjona Ferreira, J. C. 827  
 Arnal, J.-F. 566  
 Arnaud-Charra, L. 1003  
 Arndt, E. 246  
 Arner, P. 78  
 Arnold, J. F. H. 345  
 Arnolds, S. 961  
 Arnqvist, H. J. 932, 1099  
 Aroda, V. R. 806  
 Arold, G. 923  
 Aronson, J. K. 789  
 Aronson, R. 3, 807, 810, 821  
 Arora, R. 264  
 Arous, C. 402  
 Arpesella, M. 1175  
 Arroba, A. I. 94, 1118  
 Arslanian, S. 1  
 Arterburn, D. 979  
 Arvan, P. 338  
 Asadi, A. 443  
 Asahara, S.-I. 506, 533  
 Asai, A. 503, 1257  
 Asano, M. 568  
 Asghar, S. 975  
 Assaad-Khalil, S. 1070  
 Assad, N. 1094  
 Assal, J.-P. 1190  
 Assimacopoulos-Jeannet, F. 529  
 Assmann, K. E. 881  
 Asti, A. 830  
 Astiarraga, B. 583  
 Astort, F. 605  
 Astrup, A. V. 695, 697  
 Atanasova, M. A. 1084  
 Ateia, S. 877  
 Ates, C. 937  
 Athanasiadou, E. 241  
 Atkin, S. 2  
 Atkinson, M. A. 425  
 Atsumi, T. 140, 161  
 Atsumi, Y. 967  
 Attvall, S. 1052  
 Augstein, P. 424, 1047  
 Auinger, M. 1138  
 Aulinger, B. 736  
 Aurand, L. 935  
 Aureliano, M. 549  
 Avalos, G. 79, 1069, 1077, 1087  
 Avignon, A. 55, 1199, 1218  
 Avner, P. 283  
 Avogaro, A. 126, 196, 693, 1029  
 Avron, A. 455  
 Awata, T. 171  
 Awazawa, M. 734  
 Ay, C. 727  
 Ay, L. 727  
 Ayala, V. 130  
 Ayuso, E. 418, 444, 448  
 Azevedo, J. F. 987

## B

- Bělobrádková, J. 152  
 Bačová, Z. 438  
 Baan, C. A. 1022  
 Babaya, N. 650  
 Babjakova, E. 298  
 Bacci, S. 136  
 Bachaoui, M. 1070  
 Bächle, C. 322, 897, 964  
 Bachmann, M. H. 262  
 Backes, W. 1255  
 Backeström, A. 14  
 Backholer, Z. 767  
 Badawi, S. E. 370, 377  
 Bader, G. 784, 854, 862, 863, 1104  
 Bae, H. Y. 852  
 Bae, S.-J. 355  
 Baehr, V. 548, 550  
 Bagger, J. I. 334, 572  
 Bähler, L. 677  
 Bahrman, A. 997  
 Bai, J. 1222  
 Baik, S. H. 851, 852  
 Bailbé, D. 714, 887  
 Bailey, C. J. 721  
 Bailey, T. 627, 907, 1042, 1046  
 Bain, S. 802, 910, 911  
 Baines, D. 1017  
 Bairoch, A. 523



- Baixeras, E. 419  
 Baj, A. 90  
 Baker, D. 378, 379  
 Baker, G. 371  
 Bakke, S. S. 29  
 Bakker, L. E. H. 611  
 Bakker, S. J. L. 135, 155, 345  
 Bakoulas, V. 1082  
 Bakris, G. 759  
 Balavoine, A.-S. 434  
 Balde, N. M. 1013  
 Baldeon Rojas, L. 656  
 Baldi, S. 583  
 Baldin, C. 137  
 Balendran, A. 217  
 Balhuizen, A. 99, 496  
 Balis, D. 763  
 Balkau, B. 289, 300, 306  
 Balla, I. 724  
 Ballak, D. B. 66, 560, 659  
 Ballegaard, S. 978  
 Balogh, R. 1010  
 Balti, E. V. 267  
 Baltrusch, S. 43, 98, 128, 390, 395, 595, 596  
 Banciu, H. 1265  
 Bandala Sanchez, E. M. 424  
 Bang-Berthelsen, C. H. 281  
 Bani, D. 1123  
 Banke, E. 207  
 Banks, P. 772, 775  
 Banu, I. 32, 1206  
 Bar-Dayana, Y. 567, 1000  
 Barale, C. 232  
 Baranov, O. 246  
 Barata, M. G. 292  
 Barbagallo, I. 782  
 Barbanera, L. 1166  
 Barbaro, M. 675  
 Barbier, S. 1187  
 Barbosa, A. L. 652  
 Barbu, R. 607  
 Barchetta, I. 874  
 Bardelli, F. 702  
 Barg, S. 400  
 Bargellini, I. 1186  
 Barkhof, F. 167, 687  
 Barna, L. 900, 903  
 Barnard, K. 953, 1005, 1035  
 Barnard, K. 204  
 Barnett, A. H. 222, 849, 850, 955, 967, 1270  
 Baron, M. 109  
 Baroncelli, S. 1166  
 Baroni, M. G. 874  
 Barquiel Alcalá, B. 84  
 Barral, J. 1011  
 Barrett, A. 174  
 Barrio, F. 352  
 Barrio, L. C. 406  
 Barrio, P. 552  
 Barsoe, C. 1052  
 Barsotti, E. 583  
 Barsotti, M. 150  
 Bartakova, V. 152, 1128  
 Bartaskova, D. 476  
 Bartek, J. 879  
 Bartoli, N. 343, 1171  
 Bartolome, A. 533  
 Barutta, F. 655, 1133  
 Baser, O. 986  
 Basson, B. 5  
 Bastian, T. 1033  
 Bastyr, E. J. 919  
 Basu, A. 1056  
 Basu, R. 1056  
 Batch, B. 204  
 Batisse-Lignier, M. 1003  
 Battelino, T. 1  
 Battini, L. 1072  
 Battista, M.-C. 82  
 Bauduceau, B. 1106  
 Bauer, C. 259  
 Baumann, L. 1208  
 Baumann, K. 399  
 Baumgart, T. 31  
 Baustian, C. 1263  
 Bay, C. 620  
 Bazzigaluppi, E. 478  
 Bazzini, C. 478, 495  
 Beals, J. M. 24, 42, 120, 914, 918  
 Beauvieux, M.-C. 710  
 Beauwens, R. 485  
 Bech, K. 132  
 Beck, A. 768  
 Beck, R. W. 186  
 Beck-Nielsen, H. 114, 271  
 Beck-Sickinger, A. 499  
 Becker, C. 213  
 Becker, R. H. 808, 813, 814  
 Beckers, J. 635, 670  
 Beckers, L. 157  
 Bedorf, A. 736  
 Bee, Y.-M. 403  
 Beeler, N. 391  
 Beer, S. 339  
 Beffy, P. 470  
 Bego, T. 630  
 Begtrup, K. 38  
 Beguinot, F. 545, 637, 651  
 Begum, S. 1270  
 Begum, H. A. 892  
 Bekaert, M. 654, 1219  
 Belchina, J. B. 691  
 Belhadj, M. 370, 377  
 Belinova, L. 74, 75  
 Bell, M. 999  
 Bellary, S. 1038  
 Bellili-Muñoz, N. 153, 289, 300  
 Bellis, P. 830  
 Bellmann, K. 662  
 Bellomo, E. A. 101, 526  
 Belobradkova, J. 1128  
 Beltrami, A. P. 122  
 Belyaeva, N. G. 15, 941  
 Belz, M. 413, 490  
 Bem, R. 255, 256  
 Ben Hadj Slama, F. 330  
 Ben Slama, C. 1070  
 Benaiges, D. 1076  
 Benbara, A. 1094  
 BENCH-D Study Group 249  
 Bendlova, B. 1194  
 Benesch, C. 194, 196  
 Benetti, E. 660, 1123  
 Bengtsson, M. 487  
 Benito, M. 387, 533  
 Bennet, H. 99, 176, 496  
 Bennett, P. H. 1204  
 Benrubi, M. 1070  
 Bensimon, A. 1227  
 Benz, V. 673  
 Berahovich, R. 31  
 Berard, L. 944  
 Bergemann, J. 162, 501, 512  
 Bergenstal, R. M. 117, 907, 916, 917, 918, 919  
 Berggren, P.-O. 483, 1253  
 Bergis, N. 992  
 Bergman, R. N. 439  
 Bergmann, N. 978  
 Bernad, A. 419  
 Bernal, E. 164  
 Bernardi, L. 1175, 1176  
 Bernardi, S. 646  
 Bernardo, B. 541  
 Bernas, M. 236  
 Bernat-Karpinska, M. 236, 703  
 Berndt, E. 1019  
 Bernini, A. 1166  
 Berntorp, K. 1074  
 Berta, R. 583  
 Bertelli, D. 556  
 Berti, L. 299  
 Bertin, E. 730  
 Bertoin, F. 730  
 Bertolotto, A. 1072, 1093  
 Bertozzi, N. 309  
 Bertrand, G. 72  
 Bertrand, J. 1224  
 Bertuzzi, F. 478, 495  
 Besser, R. E. J. 332, 463  
 Best, J. H. 618  
 Bethel, M. 204  
 Betsholtz, C. 1124, 1212  
 Beuster, G. 590  
 Bevier, W. 1060  
 Beyer, P. 898  
 Beyer, U. 768  
 Beyerlein, A. 325  
 Bhakta, G. 1142  
 Bhargava, A. 40  
 Bhargava, P. V. 1079  
 Bhattacharya, S. 6, 148  
 Bhowmik, B. 975  
 Bi, Y.-F. 1009  
 Biagioni, M. 195  
 Bian, H. 73, 375  
 Bianchi, C. 1192  
 Bianchi, G. 90  
 Bianchi, L. 829  
 Biden, T. J. 502, 515, 517  
 Bierhaus, A. 61, 154, 1135  
 Bietiger, W. 23  
 Bijos, P. 871  
 Bilban, M. 671  
 Bilek, R. 726  
 Bilo, H. J. G. 135, 155, 345, 932, 1018, 1149  
 Biondi, G. 513  
 Birgens, H. 344  
 Birk, J. 217  
 Birkeland, K. I. 571, 882, 1071, 1226  
 Birnir, B. 489  
 Bitton, G. 118  
 Biwald, C. 825  
 Bjerre, M. 13  
 Bjerre-Christensen, U. 1164  
 Björklund, A. 893  
 Björner, J. B. 969  
 Blaak, E. 61, 709  
 Black, S. 766  
 Blaiotta, C. 883  
 Blaise, M. 442  
 Blanc, S. 1033  
 Blank, A. 737  
 Blasco-Lamarca, Y. 1103  
 Blasi, F. 637  
 Blatto, A. 1163  
 Blazquez, E. 552  
 Blevins, T. 41, 916  
 Blonde, L. 921  
 Blondeau, B. 80  
 Blouin, R. 391  
 Bluck, L. J. 96  
 Bluestone, J. A. 452  
 Blüher, M. 678, 680  
 Blum, H. 505  
 Blumensatt, M. 92  
 Blutke, A. 505  
 Boaz, M. 567, 1000  
 Bock, G. 453, 924  
 Bode, B. 38, 615, 765  
 Bodegard, J. 690  
 Boehm, B. 148  
 Boehm, K. A. 772, 773, 775  
 Boemi, M. 1020  
 Boerlin, V. 768  
 Boerman, O. C. 263  
 Boertien, W. E. 135, 155  
 Boesgaard, T. W. 297, 1201  
 Bogdanov, P. 1117  
 Bøgelund, M. 228  
 Boggi, U. 150, 437, 461, 493  
 Böhm, A. A. 299  
 Bojarska-Junak, A. 904  
 Bojsen-Møller, K. N. 10, 588  
 Bokvist, K. 547  
 Bolibar, B. 352  
 Bolinder, J. 748, 751  
 Bolla, A. M. 198  
 Bolli, G. B. 938  
 Bollyky, MD, J. 930  
 Bolognesi, L. 309  
 Bolter, L. J. 1140  
 Bolzenius, K. 316  
 Bonadonna, R. 1192  
 Bonas, S. 295  
 Bonaventura, A. 829  
 Bondar, I. A. 1134  
 Bondarenko, O. N. 1185  
 Bone, A. J. 89  
 Bonet, M. 208  
 Bongaerts, B. 1161  
 Bonifacio, E. 169  
 Bonizzoni, E. 1034  
 Bonnafous, S. 20  
 Bonnaure, H. 1265  
 Bonnet, F. 306  
 Bonnici, F. 145  
 Bonomo, K. 701, 1163  
 Bonvini, E. 146  
 Booker Porter, T. 825  
 Boomsma, D. I. 285  
 Bootsma, A. H. 731  
 Boran, G. 364  
 Borchert, A. 550  
 Borghi, C. 1234  
 Bork-Jensen, J. 599  
 Bornat, Y. 484  
 Bortolato, L. 57  
 Bortolatti, R. 555  
 Borys, S. 254, 1068  
 Bos, D. L. 263  
 Bosch, C. 1076  
 Bosch, F. 191, 418, 444, 447, 448, 633, 658  
 Boschero, A. C. 386  
 Bosco, D. 259, 408  
 Bosi, E. 198, 1034  
 Boslem, E. 517  
 Bosman, R. J. 184  
 Bosnjak, E. 95  
 Bot, S. D. M. 988  
 Botas, P. 295

- Böttcher, S. G. 912, 923  
 Böttcher, Y. 680, 694  
 Bottomley, M. J. 613  
 Bottone, P. 1072  
 Bottu, G. 511  
 Botusan, I. R. 1213, 1214  
 Botvinick, E. 264  
 Boucek, P. 435, 1152  
 Bouckenooghe, T. 81  
 Boudou, P. 80, 178  
 Bouduban, T. 20  
 Bouhassira, D. 48  
 Bouloux, P. 392  
 Boulton, A. J. M. 1178, 1184  
 Boulton, D. W. 747, 749, 757  
 Bouman, S. D. 915  
 Boumpas, D. 86  
 Bound, M. J. 820  
 Bourbon, P. 502  
 Bourdel-Marchasson, I. 845  
 Bourdin, M. 1033  
 Bourgoin, L. 639  
 Bourmeyster, N. 1122  
 Bourron, O. 1091  
 Bousaid, I. 330  
 Bouvy, M. L. 354  
 Bouzakri, K. 389, 407, 546  
 Bowe, J. E. 378, 379, 396, 440  
 Bowling, F. L. 1178  
 Boyle, K. 452  
 Bozkurt, L. 1066  
 Bozzetto, L. 76, 878  
 Bradnova, O. 726, 1194  
 Brajkovic, S. 520  
 Bramlage, P. 60  
 Brand, C. L. 37, 915  
 Brandão, V. 58, 59  
 Brandstorp-Boesen, A. 271  
 Brandt, C. 407  
 Bratina, N. 1058  
 Braun, C. 505  
 Braun, M. 486, 487  
 Brazg, R. 1040, 1042  
 Brazg, R. L. 627  
 Breitfeld, J. 680, 694  
 Brennan, L. 412  
 Brenner, M. B. 541  
 Brett, J. 806  
 Briat, A. 262  
 Briere, D. A. 547, 819  
 Brigatti, A. 1175  
 Bright, G. 821  
 Brightwell-Conrad, A. S. 593  
 Brigis, G. 699  
 Brillante, M. 990  
 Brings, S. 1210  
 Brinks, R. 347  
 Brinton, E. 1235  
 Brishoual, S. 1122  
 Brismar, K. 1213, 1214  
 Brito-Casillas, Y. 1258  
 Brix, J. M. 134, 624, 723, 727  
 Brock, C. 46  
 Brod, M. 222, 955, 967  
 Broedl, U. C. 770  
 Brogren, C. H. 262, 442, 481  
 Broholm, C. 561  
 Brom, M. 263  
 Bron, M. 628, 704, 1227, 1229, 1231  
 Brøns, C. 96, 592  
 Bronsart, L. 262  
 Brorsson, C. A. 275, 281  
 Brot-Laroche, E. 414  
 Brotons, C. 340  
 Brouwers, M. C. G. 682  
 Brouwers, O. 1255  
 Brown, A. E. 302  
 Brown, L. 302  
 Brown, S. J. 1178  
 Brubaker, P. L. 220  
 Bruce, C. 646  
 Bruce, D. G. 250, 1200  
 Brudi, P. 1236  
 Brüggemann, J. 44  
 Brugnara, L. 933  
 Bruin, J. E. 420  
 Brun, T. 523  
 Brunkwall, L. 869  
 Brunner, E. J. 356, 357  
 Bruno, G. 137, 324, 655, 1133, 1203  
 Bruttomesso, D. 194, 196, 968  
 Bruzzese, D. 945  
 Bryzinski, B. 837, 838  
 Bucciarelli, L. 336  
 Buci, L. 1165  
 Buckingham, B. A. 627  
 Buday, B. 569  
 Budreiko, O. A. 466  
 Bugliani, M. 431, 437, 461, 493  
 Buhl, M. 95  
 Buikema, H. 1126  
 Bulur, N. 485  
 Bumke-Vogt, C. 548, 550, 707  
 Bunk, M. 169  
 Burcelin, R. 566, 658  
 Burk, C. 719  
 Burkart, V. 445  
 Burke, H. 999  
 Burks, D. J. 552, 1118  
 Burnot, C. 1003  
 Burtscher, M. 1176  
 Busch, O. R. C. 65  
 Busch, S. 189  
 Buschard, K. 262, 442, 481  
 Buse, J. 800, 804  
 Busjahn, A. 260  
 Butenschoen, V. 1119  
 Butler, A. E. 425  
 Butler, P. C. 425, 538  
 Buurman, W. A. 61  
 Buxton, T. L. 34, 1262  
 Buyken, A. E. 316, 881  
 Buyschaert, M. 272  
 Buzzetti, R. 269, 896  
 Buzzigoli, E. 692  
 C  
 Cabana, R. 662  
 Cabanot-sarrau, C. 1224  
 Cabaro, S. 637  
 Cabre, J. J. 352  
 Cacciotti, L. 1180  
 Cachia, E. 45, 1154  
 Cadavez, L. 121, 539, 540  
 Cai, C. 752  
 Cai, E. P. 206, 510  
 Cai, M. 530  
 Cai, X. 994  
 Caixàs, A. 725  
 Calanna, S. 577  
 Calcaterra, V. 1175, 336  
 Caldeira, J. 555  
 Caldwell, K. 195  
 Calhoun, P. 195  
 Cali, A. 902, 938, 943, 946  
 Calle-Pascual, A. 940  
 Callejas, D. 447  
 Calvet, J.-H. 1156  
 Camacho, A. 58  
 Camara, A. 1013  
 Camastra, S. 108  
 Cambra, K. I. 166  
 Cameron, H. 1038  
 Camilleri, M. 585  
 Camillucci, M. C. 1012  
 Cammarota, S. 945  
 Campagna, G. 269, 896  
 Campbell, D. 1037  
 Campbell, L. 1184  
 Campmans-Kuijpers, M. J. E. 1022  
 Campos- Gutiérrez, B. 1103  
 Camussi, G. 1123  
 Candido, R. 646, 1020  
 Canivell, S. 12  
 Cannata, D. 706  
 Cánovas, B. 833  
 Canovatchel, W. 243, 760, 762, 763, 766  
 Cao, J. 667  
 Capaldo, B. 586  
 Capeau, J. 553  
 Capizzi, M. 269, 896  
 Capoccia, D. 874  
 Caporale, J. E. 1012  
 Caporali, A. 123  
 Capuano, G. 764  
 Carbillon, L. 1094  
 Carbone, A. 729, 735  
 Carchia, E. 513  
 Cardarelli, P. 162  
 Cardellini, M. 643, 1191  
 Cárdenas, M. 442  
 Carey, M. A. 201  
 Carey, P. E. 7  
 Caricilli, A. 555  
 Cariou, B. 39, 553, 920  
 Caritu, Y. 1033  
 Carlier, P. 1215  
 Carlier, S. 876  
 Carlin, D. 146  
 Carlotti, F. 433  
 Carlsson, A. 278  
 Carlsson, P.-O. 261  
 Carlsson, S. 168, 270, 317, 320  
 Carmignani, A. 1072  
 Carmody, L. 79, 1064, 1069, 1077  
 Carnelli, F. 122  
 Caro, A. 484  
 Carpegiani, C. 692  
 Carr, B. 364  
 Carr, R. D. 828  
 Carrera, M. 1076  
 Carrette, O. 1058  
 Carrozzi, G. 309  
 Carstensen, B. 700  
 Carstensen, M. 103, 294  
 Carvalho, A. C. 1225  
 Carvalho, E. 549, 557, 1169  
 Casadiegos, S. 1169  
 Casagrande, V. 64, 159, 160, 1191  
 Casali, C. 124  
 Casamitjana, R. 479  
 Casaña, E. 444  
 Casellas, A. 418, 444, 447, 448  
 Casey, R. 999  
 Cash, T. 1154, 1181  
 Casini, P. 539, 540  
 Casiraghi, F. 711  
 Cassel, R. 532  
 Castanas, E. 86  
 Castañeda, J. 1058  
 Castaño, C. 144  
 Castano, L. 1015  
 Castano, L. 963  
 Castell, C. 352  
 Castells, I. 991  
 Castillo, K. 322, 897, 964  
 Castro, C. 603  
 Castro, E. 833  
 Castro, M. C. 883  
 Castro Duforny, I. 84  
 Catalan, V. 562  
 Cataldo, D. 104  
 Catapano, A. L. 945  
 Catargi, B. 1224  
 Catoire, M. 598  
 Catrina, S.-B. 1213, 1214  
 Catterall, W. A. 483  
 Cau, V. 324  
 Cauley, J. A. 674  
 Causevic, A. 630  
 Cavalcanti da Silva, S. 966  
 Cavallera, M. 64, 160  
 Cavallo, G. 1192  
 Cavallo, M. G. 874  
 Cavallo-Perin, P. 137, 655, 1133, 1163  
 Cavalot, F. 232, 701, 1139, 1192  
 Cavan, D. A. 953, 982, 1005, 1035  
 Cavey, G. S. 593  
 Ceccarelli, E. 1085  
 Cederholm, J. 51, 54, 182  
 Cefalu, W. T. 745, 756, 760, 762, 763  
 Cejas, J. 603  
 Ceponis, J. 965  
 Ceredig, R. 1263  
 Ceriello, A. 1034  
 Ceriotti, F. 198  
 Cerrillos, L. 1061  
 Cersosimo, E. 245  
 Cervelli, R. 1186  
 Cha, B. 1177  
 Chad, A. 27, 130  
 Chaieb, L. 330  
 Chaieb, M. 330  
 Chaignepain, S. 397  
 Chaimy, T. 1000  
 Chakera, A. J. 53, 358  
 Chalamandaris, A.-G. 242, 755  
 Chamas, L. 629  
 Chambon, P. 566  
 Chan, D. T. M. 1220  
 Chan, J. Y. 515  
 Chana, G. 1090  
 Chander, A. 396  
 Chandru, C. 793  
 Chang, C. T. 1  
 Chang, X. 73  
 Chantry, M. 321  
 Chanu, B. 1199, 1206, 1215  
 Charbonneau, A. 662  
 Charlton, P. 47  
 Charnaux, N. 32  
 Chatgililoglu, C. 504  
 Chatterjee, D. J. 1  
 Chatzi, L. 86  
 Chatzigeorgiou, A. 158, 663  
 Chaumet, G. 1266  
 Chauvin, M.-A. 532, 667  
 Chavakis, T. 158, 663  
 Chavez, A. 791

- Chazot, P. L. 1123  
 Cheetham, S. C. 771  
 Chen, A. 1183  
 Chen, E. 1235  
 Chen, J. 752  
 Chen, K. 1227  
 Chen, L. 1254  
 Chen, M. 110  
 Chen, M. 1153  
 Chen, R. 1187  
 Chen, S. 5, 781, 798  
 Chen, S. 631  
 Chen, T. 836  
 Chen, V. 186  
 Chen, W. J. Y. 1219  
 Chen, X. 1263  
 Cheng, C. C. 25, 819  
 Cheng, D. 1227  
 Cheng, G. 881  
 Cheng, Y.-C. 284  
 Cherifi, B. 876  
 Chermak, F. 876  
 Cherrington, A. D. 24, 37, 224  
 Cheta, D. 877  
 Cheung, A. T. 443  
 Chicano, A. 870  
 Chiheb, S. 32, 1206, 1215  
 Chillaron, J. 1076  
 Chin, H.-J. 891  
 Chin, J. 58, 59  
 China DiaSTAGE Group 349, 836  
 Ching, A. 747  
 Chinnery, P. F. 302  
 Chirio, M. 232  
 Chiswell, K. 204  
 Chlup, R. 879  
 Cho, K. 161  
 Cho, L. 361, 1187  
 Cho, M. 815  
 Cho, Y.-W. 1242  
 Choi, B. 264  
 Choi, S. L. 913, 922  
 Choi, Y.-J. 1008, 1242  
 Chondhary, P. 441  
 Chong, B. Y. 1210  
 Chorepsima, S. 1241  
 Chou, D. H. C. 494  
 Chou, E. 803, 980  
 Choudhary, A. 494  
 Choudhary, P. 1027  
 Choukem, S. P. 80  
 Chow, F. 581  
 Chow, W. 1031  
 Chowdhury, H. 1078  
 Chowdhury, S. 873  
 Christ, A. D. 768  
 Christensen, T. E. 957  
 Christensen, U. B. 253  
 Christiaen, D. 262  
 Christiansen, C. 114  
 Christiansen, E. 382  
 Christiansen, J. S. 13, 926  
 Christiansen, M. 1042, 1046  
 Chu, P.-L. 920  
 Chubb, S. A. P. 1200  
 Chumak, S. O. 466  
 Chung, C. H. 852  
 Chung, K.-J. 158  
 Ciaccio, S. 968  
 Ciangura, C. 1091  
 Ciaraldi, T. P. 244  
 Ciccarelli, L. 729  
 Cicero, A. F. G. 735, 829  
 Cid-Ruzafa, J. 373  
 Cifarelli, V. 235  
 Cignarelli, A. 513, 675, 1260  
 Cikomola, J. C. 1268  
 Cimino, E. 968  
 CIMT Trial Group 1201  
 Cinek, O. 88  
 Cioni, R. 1186  
 Cipriano, P. 76  
 Cirincione, B. 825  
 Citarella, A. 945  
 Citko, A. 689  
 Cito, M. 124  
 Ciudin, A. 1112, 1267  
 Clapham, L. 613  
 Clare-Salzler, M. 268  
 Clark, B. 193  
 Clausen, T. D. 1083  
 Claussnitzer, M. 287  
 Cleasby, M. E. 591, 668  
 Cleland, J. 821  
 Clément, S. 639  
 Clemente-Casares, X. 451  
 Clerici, G. 1171  
 Cnop, M. 511  
 Coates, J.-A. 7  
 Cobbaert, C. 1149  
 Cobble, M. 966  
 Cobelli, C. 194, 585, 1056  
 Cocker, D. 1014  
 Cocozza, S. 878  
 Coester, H.-V. 909  
 Cohen, G. 504  
 Cohen, O. 1058  
 Cohen, R. V. 9  
 Colagiuri, S. 617  
 Colca, J. R. 593  
 Cole, S. L. 593  
 Coleman, K. 979  
 Colhoun, H. M. 239  
 Colle, B. 1266  
 Colleran, K. M. 1021  
 Collino, M. 660, 1123  
 Collins, F. S. 174  
 Collombat, P. 415  
 Collura, N. 602  
 Colombo, M. 90  
 Colvin, A. E. 1030  
 Comaschi, M. 57  
 Comassi, M. 291  
 Commuzzie, A. 711  
 Competence Network Diabetes Mellitus 322  
 Conde, S. V. 93, 554  
 Conget, I. J. 479, 1104  
 Conley, A. 457  
 Conrad, R. 619  
 Conradsen Hiort, L. 939  
 Consoli, A. 1249  
 Constantine, G. R. 1097  
 Contag, C. H. 262  
 Conti, M. 664  
 Cook, M. 1041  
 Cook, W. 837, 838  
 Cooney, G. J. 591, 638, 649  
 Cooper, D. E. 602  
 Cooper, G. 1178  
 Cooper, G. J. S. 1210  
 Cooper, J. G. 910, 911  
 Cooper, M. E. 36, 646  
 Cooper, R. A. 1140  
 Coppelli, A. 1186  
 Coppo, E. 1163  
 Corbelli, A. 1133  
 Cordeiro, L. H. L. 604  
 Cordella, D. 122  
 Cornejo, D. 382  
 Cornejo, M. 1141  
 Corominas, E. 1076  
 Corpas, M. 870  
 Corraliza, L. 1112, 1117  
 Correa, J. 9  
 Correale, L. 1175  
 Correig, X. 684  
 Cortés, M. 612  
 Cortes Rodriguez, M. 293  
 Cosentino, N. 1221  
 Coskun, T. 25  
 Cosner, C. 502  
 Cosson, E. 32, 116, 1094, 1199, 1206, 1215  
 Costa, B. 352  
 Costa, O. 267  
 Costa, S. 1061  
 Costa, S. 968  
 Costabile, G. 878  
 Costagliola, L. 76  
 Costantino, C. 874  
 Coste, O. 1266  
 Costes, S. 538  
 Costet, P. 553  
 Cotugno, M. 586  
 Coudert, L. 397  
 Coughlan, M. T. 151  
 Courrèges, J. 2, 1265  
 Courson, A. 1266  
 Courtney, M. 415  
 Couto, J. Márcio. 604  
 Covey, S. D. 570  
 Coward, R. J. 1125  
 Cox, A. 547  
 Cramb, R. 984  
 Cranston, I. 953, 982, 1005, 1035  
 Crea, F. 1221  
 Creane, M. 1172, 1263  
 Cree, L. M. 302  
 Cristina, P. 1198  
 Cromie, D. 187  
 Crossman, R. 1017  
 Crouzet, M. 730  
 Crowder, A. F. 1142  
 Crowe, C. 1077  
 Cruccu, G. 48  
 Crutzen, R. 485  
 Cruzado, M. 603  
 Cucinotta, D. 1034  
 Cuesta, A. 419  
 Cui, S. 818  
 Culita, E. 607  
 Cullinan, J. 1095  
 Cunha-Neto, E. 404  
 Cunningham, A. 999  
 Cunningham, S. G. 990  
 Currie, A. 995  
 Curtis, B. 954  
 Cussac Pillegand, C. 32, 1094  
 Cutler, Jr., G. B. 42  
 Cutolo, P. Paolo. 586  
 Cyganek, K. 1026, 1065, 1068  
 Cymeryng, C. B. 605  
 Cynshi, O. 768  
 Czech, A. 17, 236, 703  
 Czupryniak, L. 928, 931, 934, 947
- D**
- D'Agostino, R. Jr. 1238  
 D'Alessandro, A. 830  
 D'Alessandro, G. 830  
 D'Alessio, D. 802  
 D'Amario, D. 1221  
 D'Amato, C. 1180  
 D'Angelo, A. 735  
 D'Esposito, V. 651  
 D.E.S.I.R. Study Group 289  
 Dabbous, O. 628  
 Dabelea, D. 1238  
 DaCosta, C. 774  
 Dagan, S. 145, 455  
 Dahl-Jørgensen, K. 1162  
 Dahlman, I. 78  
 Daida, H. 318  
 Daifotis, A. G. 146  
 Dailey, G. 935, 943  
 Dain, M.-P. 938, 946, 947  
 Daives, J. 1154  
 Dalakleidi, K. 1080  
 Dalal, M. R. 797, 981  
 Dalbøge, L. S. 177  
 Dalfra, M. 1085  
 Dalla Man, C. 1056  
 Dalle, S. 72  
 Dallinga-Thie, G. M. 65, 644  
 Dam, S. S. 1111  
 Dambrova, M. 1256  
 Damdindorj, B. 405  
 Damm, P. 83, 1083  
 Dammeier, S. 542  
 Danassi, D. 507  
 Dang, T. 31  
 Daniel, H. 664, 717  
 Daniel, W. G. 997  
 Daniele, G. A. 291, 791  
 Daniels, M. 1110  
 Daniels, S. 1238  
 Danielson, M. E. 674  
 Danne, T. 902, 946  
 Das, S. 1089  
 Dassau, E. 1060  
 Dattani, A. A. 440  
 Daures, J. 55, 1218  
 Davalli, A. M. 478, 495, 711  
 Davato, F. 643, 1191  
 Davé, S. 373  
 Davidson, L. E. 587  
 Davies, J. 45, 1181  
 Davies, M. J. 110, 834, 985  
 Davies, M. J. 311, 621, 764  
 Davis, K. L. 797, 936, 981  
 Davis, T. M. E. 250, 1200  
 Davis, W. A. 250, 1200  
 Day, W. W. 697  
 Dayan, C. 456  
 Dayeh, T. 105, 142  
 de Araújo, T. M. 652  
 De Arujo, E. P. 556  
 de Beaufort, C. E. 963, 1015  
 De Bellis, A. 1166  
 De Bernardinis, M. 874  
 De Block, C. 267, 1058  
 de Bruin, T. 754  
 de Cillia, V. A. M. 411  
 De Cosmo, S. 136, 1196  
 De Cré, M. 1014  
 De Fazio, M. 675  
 De Fronzo, R. 711  
 De Gaetano, A. 351  
 de Galan, B. E. 929  
 de Geus, E. J. 285  
 de Graaf, N. 433  
 de Hollanda, A. M. 479  
 de Koning, E. J. P. 433, 499  
 De la Fuente, J. L. 1012



- de la Villa, P. 191, 1118  
 De Laurenzi, V. 1249  
 de Ledinghen, V. 876  
 De León Rodríguez, D. 1190  
 De Lonlay, P. 414  
 De Luca, L. 945  
 De Marco, A. 1249  
 de Marinis, Y. 530  
 De Meyts, P. 218  
 de Portu, S. 1058  
 de Roos, A. 1219  
 De Simone, G. 57  
 de Sonnaville, N. 65  
 De Souza, E. 906  
 De Tata, V. 470  
 De Toro-Martín, J. 417, 528  
 de Vos, W. M. 65, 644  
 De Vries, J. H. 184  
 de Zeeuw, D. 764  
 Deacon, C. F. 246, 248, 582  
 Dear, A. E. 780  
 DeBarbieri, G. 1175  
 DeBruin, T. 745, 756  
 Decochez, K. 267  
 Decoudier, B. 730  
 Deeg, E. 60  
 Deeg, M. 112, 818  
 Deelman, L. E. 1126  
 Defrance, F. 434  
 DeFronzo, R. A. 245, 563, 564, 747, 791  
 Degerman, E. 207  
 Degrell, P. 1144  
 DeHennis, A. D. 1030  
 Dei Cas, A. 124  
 Deijen, J. B. 687  
 Deinhard, J. 940  
 Dejager, S. 845  
 Dejgaard, T. F. 1083  
 Dekker, J. M. 133, 234, 285, 354, 988, 1002  
 Dekker Nitert, M. 107, 142  
 Del Prato, S. 40, 291, 615, 856, 1072, 1093, 1192, 1196  
 Dela, F. 132, 599  
 Delemer, B. 730  
 Delenne, B. 1224  
 Delgado, E. 295, 658  
 Della Corte, G. 878  
 Della Pepa, G. 878  
 Deller, S. 924  
 Delli, A. J. 278  
 Delplanque, J. 81  
 Demeester, S. 267  
 den Engelsen, C. 868  
 Deng, L. 766  
 Deng, W. 73  
 Denroche, H. C. 570  
 DePaoli, A. M. 587  
 Derevyanko, O. S. 465  
 Derks, K. 656  
 Dermitzakis, E. T. 139  
 Derosa, G. 729, 735, 829  
 Derragui, A. 1269  
 Desai, J. R. 353  
 Desai, M. 764  
 Desbiez, F. 1003  
 Desborough, L. 1050  
 Dessapt-Baradez, C. 1209  
 Deville, L. 178  
 DeVries, J. H. 38, 194, 196  
 Dezaki, K. 405, 488  
 Dezortova, M. 74  
 Dhadda, P. K. 432, 440  
 Dharmalingam, M. 40, 851, 852  
 Dhir, M. 1099  
 Di Bartolo, V. 782  
 Di Cairano, E. S. 478, 495  
 Di Cianni, G. 1093  
 Di Domenico, D. 457  
 Di Gennaro, F. 1180  
 Di Gruccio, J. M. 605  
 Di Martino, L. 701  
 Di Palma, F. 194  
 Di Pietro, N. 1249  
 Di Pino, A. 577  
 Di Terlizzi, G. A. 198  
 DIA-AID 1 Study Group 145  
 DIAdvisor Consortium 1029  
 Diago Cabezudo, J. 1038  
 Diakanastasis, B. 21  
 Diamant, M. 167, 609, 687, 800, 1219  
 Diamantis, T. 724  
 Dias, A. 1169  
 Dibner, C. 259  
 Dicembrini, I. 343  
 Dick, E. 711  
 Dicken, H.-D. 679  
 Dickie, P. 443  
 Diéguez, C. 562  
 Diekmann, U. 215  
 Diemar, S. S. 138  
 Dierick-Gallet, A. 1091  
 Dietert, G. 737  
 Dietrich, M. G. 388  
 DiGenio, A. 797, 981  
 Dimcevski, G. 46, 47  
 Dimitriadis, G. 1240  
 Ding, L. 454  
 Ding, Z.-M. 774, 776  
 Diniz, J. 1225  
 Dinneen, S. F. 999, 1188  
 Dirice, E. 175  
 Dirksen, C. 10, 588  
 Dirkx, R. 410  
 Dixon, J. 719, 720  
 Djurhuus, C. B. 910  
 Docherty, K. 429  
 Dockery, P. 1172, 1263  
 Dogar, H. 264  
 Doi, A. 686  
 Dokas, J. 26, 27  
 Dolan, L. 1238  
 Dolderer, J. H. 683  
 Dolezalova, K. 726  
 Doll, W. 194  
 Dollet, L. 553  
 Dominguez, C. 819  
 Dominguez-Bendala, J. 419  
 Dommergues, M. 1091  
 Domsgen, E. 162, 467  
 Dong, H. 799  
 Dong, Y. 315  
 Donovan, M. 837, 838  
 Donsmark, M. 800  
 Dooley, S. 707  
 Dorai, A. 875  
 Dorau, M. 737  
 Dorchy, H. 267  
 Dores, J. M. 1088  
 Doria, A. 136  
 Dorkhan, M. 270  
 Doronzo, G. 232  
 Dotta, F. 5, 104, 431, 468, 781, 1085  
 Dotzauer, A. 467  
 Douard, H. 1224  
 Doucet, J. 1106  
 Doyon, M. 82  
 DPV Initiative and the BMBF Competence Networks Diabetes and Obesity 314  
 Dragomir, A. 877  
 Dragoumanos, V. 16  
 Dragut, R. 877  
 Dreher, W. 501  
 Drent, M. L. 687  
 Dreval, A. V. 792  
 Drewes, A. M. 46  
 Drows, G. 382  
 Drexel, H. 339  
 Drexhage, H. A. 656  
 Dreyer, N. A. 719  
 Driessen, A. 157  
 Drion, I. 155, 1018, 1149  
 Druelle, N. 415  
 Druet, C. 321  
 Dry, S. M. 425  
 Duan, H. 817  
 Duarte, R. 940  
 Dubb, K. 1270  
 Dubois, S. 532  
 DuBois, S. L. 25  
 Dubsky, M. 255, 256  
 Dubuis, G. 516  
 Ducreux, S. 532  
 Duda-Sobczak, A. 227, 971  
 Due, P. 963  
 Due-Christensen, M. 1016  
 Duennwald, T. 1176  
 Düfer, M. 382  
 Duffy, A. 1263  
 Dugas, J. 1033  
 Dugi, K. 1006, 1007  
 Duhamel, D. 523  
 Dujic, T. 630  
 Dulak, J. 254  
 Dunbar, J. D. 120  
 Dunbar, P. 350  
 Dunger, D. B. 96, 195  
 Dunlap, S. M. 235  
 Dunn, J. T. 728  
 Dunne, E. 79, 1064, 1069, 1077, 1087, 1095  
 Dunne, N. 993  
 Dünnebeil, J. 694  
 Dunseath, G. 983  
 Dunstheimer, D. 898  
 Dupin, J. 1156  
 Duque, M. F. 1169  
 Durán-García, S. 6  
 Durand, A. 667  
 Düring, M. 2, 826  
 Durkan, M. 1064  
 Duteil, S. 1215  
 Dutta, D. 873  
 Dvorakova, K. 726, 1194  
 Dworak, M. 784, 854  
 Dyachok, O. 68  
 DYDA Investigators 57  
**E**  
 Eayres, D. 341  
 Ebisui, O. 973  
 Echouffo-Tcheugui, J. B. 183  
 Eckardt, K. 669  
 Eckel, J. 654, 669  
 Eckman, M. 979  
 Edalat, A. 382  
 Edelman, S. V. 993  
 EDGE Steering Committee 863, 1104  
 Edgerton, D. S. 37  
 Edmonds, A. 1230  
 Eeg-Olofsson, K. 51, 54, 182  
 Eekhoff, E. M. 285  
 Efimov, A. S. 691  
 Eggleston, K. 1019  
 Ehlers, M. 452  
 Ehlers, M. R. 147, 454  
 Ehrmann, D. 992  
 Eickelmann, P. 398  
 Eisenbarth, G. 106, 268  
 Eizirik, D. L. 511  
 Ejlskjær, N. 47, 1160  
 Ekelund, M. 1074  
 Ekelund, M. 11  
 El Bache, N. 1070  
 El Ghomari, H. 1070  
 El Hefnawy, M. M. F. 905  
 El Ouamari, A. 175  
 Elding-Larsson, H. 278  
 Eldrup, E. 1160  
 Eleftheriadou, I. 1109  
 Elgart, J. F. 1012  
 Elgzyri, T. 107  
 Elias, D. 145, 455  
 Elias, I. 633  
 Eliasson, B. 51, 54, 182  
 Eliasson, L. 97, 486  
 Ellard, S. 332  
 Ellenbroek, J. H. 433  
 Elleri, D. 195  
 Ellervik, C. 344  
 Elliott, J. 225  
 Ellison, J. 1038  
 Elsner, M. 514  
 Emanueli, C. 122, 123  
 Emery, N. 153, 289, 300  
 Emilia, R. 1198  
 Emral, R. 937  
 Emser, A. 36, 847, 850  
 Emslie-Smith, A. 990  
 Enache, G. 877  
 Enax, S. 962  
 Endahl, L. 39, 615, 921, 949  
 Endres, J. 439  
 Engel, D. 157  
 Engel, S. S. 827, 834, 985  
 Engelen, L. 133  
 Engeltgau, M. 1204  
 Engelse, M. A. 433  
 Engkilde, K. 481  
 Enguix, N. 127  
 Engwerda, E. E. C. 929  
 Enigk, B. 680  
 Epstein, O. I. 887  
 Erästö, P. 616  
 Erdmann, E. 787, 1228  
 Eren, R. 145, 455  
 Ericson, U. C. 288, 290, 869  
 Eriksen, E. F. 571  
 Eriksson, B. 261  
 Eriksson, I. 373  
 Eriksson, J. 261  
 Eriksson, J. W. 549  
 Eriksson, K.-F. 107  
 Eriksson, O. 261  
 Eriksson, S. 14  
 Ermácóra, M. 410  
 Ertl, L. 31  
 Escribano, O. 387  
 Escrivá, F. 417, 528  
 Esguerra, J. L. S. 486

- Eskelinen, J. J. 601  
 Esmatjes, E. 12, 479  
 Espes, D. 261  
 Esposito, V. 815  
 Essermeant, L. 812  
 Esteban, Y. 645  
 Esteve, E. 676  
 Esteves, G. 603  
 Eto, H. 219  
 Etoh, H. 506  
 Eurich, D. T. 52, 329  
 Eury, E. 81  
 Evans, M. 228  
 Evans, M. 626  
 Evans, M. L. 576, 622  
 Exiara, T. 1157
- F**
- Faber, J. 138, 978  
 Fabianova, M. 298  
 Fabre, A. 566  
 Fabris, B. 646  
 Fabrizi, M. 64, 159, 160  
 Fadini, G. P. 126, 693  
 Færch, K. 96, 356, 357  
 Færch, L. H. 279  
 Fagerholm, V. 1261  
 Faglia, E. 123  
 Fagot-Campagna, A. 321  
 Fajmon, M. 200  
 Fanelli, C. G. 938  
 Fantozzi, R. 660, 1123  
 Farghaly, H. S. M. 436  
 Faria, C. C. 604  
 Faria, J. A. 652  
 Farmer, A. J. 788, 789  
 Farmer, B. 224  
 Farré, A. 612  
 Farrera-Sinfreu, J. 1112  
 Farret, A. 194, 1029  
 Faruque, M. O. 681, 892  
 Fasoulaki, M. 724  
 Faurite, B. 415  
 Faustman, D. L. 464  
 Favaro, E. 1163  
 Febbraio, M. 646  
 Federici, M. 64, 159, 160, 643, 1191, 1249  
 Federico, G. 90  
 Feige, J. 1224  
 Feigh, M. 114  
 Fejfarová, V. 255, 256  
 Felace, G. 1020  
 Feldman, D. 118, 119  
 Feliu, J. E. 1193  
 Fels, J. J. 915  
 Felton, A. M. 966  
 Fendler, W. 1237  
 Feng, Y. 1119  
 Feng, Z. C. 393, 416  
 Fenzl, A. 411  
 Fernandez, R. 436  
 Fernández, S. 387  
 Fernandez Ruiz, R. 394  
 Fernández-Carneado, J. 1112  
 Fernández-Millán, E. 94, 417, 528  
 Fernandez-Perez, A. 508  
 Fernández-Real, J. M. 208, 295, 562, 658, 676  
 Fernández-Rebollo, E. 286  
 Ferrannini, E. 108, 306, 583, 721, 761  
 Ferraro, M. A. 729, 829  
 Ferré, T. 633  
 Ferreira, I. 133, 1239  
 Ferreira, S. M. 386  
 Ferreri, C. 504  
 Ferretti, E. 104  
 Fertuso, G. 830  
 Feskens, E. J. 1239  
 Festa, A. 5, 781  
 Fetita, L. S. 80  
 Fetita, S. 178  
 Fex, M. 99, 176, 496  
 Fica, S. 342  
 Ficarella, R. 513, 675  
 Fiera, F. 903  
 Figueiredo, H. 436, 684  
 Figueroa, K. 759  
 Filipova, N. V. 466  
 Filippini, F. 437, 461, 493  
 Finan, D. A. 1057  
 Fingerlin, T. E. 106, 268  
 Finn, R. P. 53  
 FinnDiane Study Group 156, 170, 327, 1135, 1137, 1176  
 Finner, H. 103, 294  
 Finnish Paediatric Diabetes Register 274, 277  
 Fino, E. 478  
 Finzi, G. 711  
 Fiorentino, T. V. 791  
 Fiory, F. 545  
 Fischer, D. 979  
 Fischer-Rosinsky, A. 694  
 Fishbach, M. 1224  
 Fisher, H. 621  
 Fisher, L. K. 454  
 Fitzgerald-Miller, L. 457  
 Flanagan, S. E. 53  
 Platt, P. R. 579  
 Fu, A. Z. 985  
 Fu, H. 112, 818  
 Fu, M. 243  
 Fuellen, G. 449  
 Fugmann, M. 287  
 Fujihara, K. 1217, 1243  
 Fujimaki, K. 865  
 Fujimaki, R. 335, 337  
 Fujimori, N. 140  
 Fujimoto, H. 718  
 Fujimoto, K. 733  
 Fujimoto, M. 1124, 1212  
 Fujimura, T. 525  
 Fujioka, K. 589, 927  
 Fujishiro, M. 734  
 Fujita, Y. 443, 578  
 Fujita, Y. 648, 708  
 Fujitani, Y. 865  
 Fujiwara, S. 219, 885  
 Fukatsu, A. 740, 741  
 Fukuda, H. 301  
 Fukui, K. 469  
 Fukui, M. 568  
 Fukushima, T. 648, 708  
 Fukushima, Y. 767  
 Fulcher, G. 764  
 Fumeron, F. 153, 289, 300  
 Funahashi, T. 282  
 Funakubo, M. 1107  
 Fung, A. 765  
 Funk, B. 384  
 Furber, S. 800, 802  
 Furhmann, A. 557  
 Fürsinn, C. 411  
 Furukawa, S. 973  
 Furukawa, T. 335  
 Furuta, H. 686, 1147  
 Fusco, G. 704, 1229, 1231  
 Fysekidis, M. 116
- G**
- Gabert, L. 209  
 Gabriel, R. 340  
 Gabriela, R. 1198  
 Gæde, P. 1223  
 Gaens, K. 61  
 Gaffney, G. 1077  
 Gaffney, P. 364  
 Gaggini, M. 291, 584, 692  
 Gagliardi, L. 1165  
 Gagliardino, J. J. 883, 1012  
 Gagniac, P. 280  
 Gaitan, J. 484  
 Galbo, T. 19  
 Galbo-Jørgensen, C. B. 228  
 Galimova, I. R. 1062  
 Gall, M.-A. 926  
 Gall, W. 108, 563, 564  
 Gallagher, E. J. 706  
 Gallas, J.-F. 812  
 Gallcher, T. 1064  
 Gallen, I. 1014  
 Gallwitz, B. 5, 374, 781  
 Gamble, J.-M. 52  
 Game, F. L. 1168  
 Gandasi, N. 400  
 Gandhi, P. 851, 852  
 Gandhi, R. 45, 1181  
 Gansevoort, R. T. 135, 155  
 Gantz, I. 110  
 Gao, J. 1153  
 Gao, J. 143  
 Gao, S. 587  
 Gao, X. 73, 312, 375, 1153  
 Gao, Z. 641  
 Garber, A. J. 40, 617  
 Garcia, A. 1051  
 Garcia, A. 436, 500  
 Garcia, A. G. 1179  
 Garcia, G. 387  
 Garcia, M. 633  
 Garcia, N. 1061  
 Garcia Almeida, J. M. 293  
 García-García, B. 1103  
 Garcia-Arcos, I. 397  
 Garcia-Arumí, J. 1117  
 Garcia-Escobar, E. 672  
 Garcia-Ramírez, M. 1112  
 García-Rovés, P. M. 394  
 Garcia-Sanz, P. 508  
 Garcia-Serrano, S. 672  
 Garduno-Garcia, J. 245  
 Garg, S. 41, 627, 1042  
 Garhyan, P. 112, 818  
 Garofolo, M. 1196  
 Garrow, A. P. 1188  
 Garten, A. 499  
 Gasa, R. 500, 539  
 Gaspari, T. 780  
 Gastaldelli, A. 291, 310, 478, 564, 584, 692, 791  
 Gately, M. 999  
 Gatterer, H. 1176  
 Gattesco, S. 214  
 Gaudet, P. 523  
 Gault, V. A. 579  
 Gause-Nilsson, I. 745, 756  
 Gautier, J.-F. 80, 178  
 Gavra, P. 56  
 Gawrecki, A. 227

- Gazis, A. 1216  
 GDS Group. 44  
 Gedulin, B. 820  
 Gee, M. E. 350  
 Geelhoed-Duijvestijn, P. H. L. 731  
 Gehrman, W. 514  
 Geijselaers, S. L. 1239  
 Geiser, J. S. 914  
 Gelmont, D. M. 147  
 Genevieve, M. 1246  
 GENFIEV-FoRiSID Study Group 1192  
 Gennari, L. 1085  
 Genova, M. G. 1084  
 Genové, G. 1212  
 Genovese, S. 269, 336, 1171  
 Gensini, G. 1171  
 Gentile, S. 249  
 Gentileschi, P. 643  
 Geoghegan, R. 999  
 Georgiana, E. 1198  
 Georgiou, V. 86  
 Gerber, P. A. 526  
 Gerdes, N. 157  
 Gerdtham, U. 1052  
 Gerich, J. 815  
 German Competence Network Diabetes Mellitus 897, 898  
 German Pediatric Surveillance Unit (ESPED) 322, 964  
 Germe, M. 980  
 Gerodiab Group 1106  
 Gesing, J. 678  
 Ghezzi, G. 137  
 Ghio, A. 1072, 1093  
 Ghiraldini, F. G. 653  
 Ghirlanda, G. 1221  
 Ghosh, A. 244  
 Ghosh, S. 682, 873  
 Giaccari, A. 269  
 Giacco, A. 76  
 Giacco, R. 878  
 Giani, G. 310, 322, 347, 784, 854, 897, 964  
 Giannella-Neto, D. 604  
 Giannopoulou, E. 169  
 Gier, B. 425  
 Giesler, P. D. 585  
 Giet, O. 1011  
 Giglio, V. 782  
 Gilbert, N. 653  
 Gillard, P. 267  
 Gillberg, L. 297  
 Gillespie, P. 1095  
 Gillson, C. 173  
 Gilon, P. 101  
 Giménez, M. 479  
 Gimenez-Perez, G. 991  
 Gimeno-Orna, J. A. 1103  
 Gin, H. 710, 876, 1246  
 Giorda, C. B. 57, 1020  
 Giorgino, F. 125, 269, 513, 675, 1034, 1192, 1260  
 Giotaki, E. 474  
 Girelli, A. 968  
 Gitelman, S. E. 452, 454  
 Gitt, A. K. 60  
 Giubilato, S. 1221  
 Giugliano, D. 940  
 Giunti, S. 655  
 Gjernes, E. 415  
 Glettler, K. 924  
 Glindorf, M. 226  
 Globe, D. 719, 720  
 Gloyn, A. L. 174  
 Glumoff, V. 473  
 Glynn, L. G. 1188  
 Gnudi, L. 124, 793, 1209  
 Gošcik, J. 689  
 Göbl, C. S. 1066  
 Goday, A. 1076  
 Göke, B. 736  
 Gelay, A. 1190  
 Goldenberg, R. 810  
 Goldstein, B. J. 110, 827, 834  
 Goletzke, J. 316  
 Golm, G. T. 827, 834  
 Golstein, P. E. 485  
 Gomez-Ambrosi, J. 562  
 Gómez-Hernández, A. 387  
 Gómez-Zumaquero, J. 870  
 Gomis, R. 286, 394, 436, 500, 645, 684  
 Goncalves Jr, I. 1225  
 Gong, D. 1210  
 Gong, Q. 1204  
 Gong, Y. 847  
 Goñi, M. J. 166  
 González, A. 127  
 Gonzalez, C. 554  
 Gonzalez, C. 710, 876, 1246  
 Gonzalez, L. 1012  
 González, N. 634  
 González-Clemente, J. M. 725  
 Gonzalez-Galvez, G. 951  
 González-Nieto, D. 406  
 González-Rodríguez, Á. 94, 1118, 1125  
 Gonzalez-Ruano, E. 645  
 Goodpaster, B. H. 674  
 Goossens, G. H. 61  
 Gorbunov, E. A. 887  
 Gorczynska - Kosiorz, S. 657  
 Gordin, D. 327, 1176  
 Goretti, C. 1093  
 Görgens, S. W. 669  
 Goring, S. M. 746  
 Gorska, M. 1086, 689  
 Górski, A. 703  
 Gorski, T. 499  
 Gorter, K. J. 1022  
 Gorus, F. K. 267  
 Gosset, P. 81  
 Gotlib, S. 181  
 Goto, S. 1108  
 Gotthardt, M. 263  
 Gotti, A. 122  
 Gottlieb, P. A. 147, 452, 454, 457  
 Gottschalk, M. 454  
 Gough, A. 1023  
 Gough, S. C. L. 615, 617  
 Gourdy, P. 566  
 Goya, L. 528  
 Gozzo, E. 1165  
 Grabert, M. 307  
 Grady, M. 1037  
 Graham, A. M. 1259  
 Grallert, H. 169  
 Grammatikou, S. 867  
 Grand, T. 414  
 Grandy, S. 755, 777  
 Grange, C. 1123  
 Graninger, W. 453  
 Grant, D. 956  
 Grant, P. S. 972  
 Grassi, A. 1163  
 Grassi, G. 968, 1163  
 Grassl, G. A. 443  
 Graw, J. 505  
 Greco, C. 1180  
 Green, B. 984  
 Green, J. B. 204  
 Green, L. 1027  
 Greenbaum, C. 452, 930  
 Greer, J. 774, 776  
 Greer, J. 993  
 Gregersen, B. 1036  
 Gregersen, S. 296, 666  
 Gregg, E. 1204  
 Grempler, R. 769, 771  
 Gretz, N. 189, 707  
 Greulich, S. 92, 1219  
 Greve, J. M. 61  
 Gribble, F. M. 576, 716  
 Griebel, T. 511  
 Grieco, F. A. 104, 431, 468  
 Griffen, S. C. 747, 749, 757  
 Griffin, M. 1263  
 Griffin, P. 799  
 Griffin, S. J. 183  
 Griffo, E. 586  
 Grigoropoulou, P. 1109  
 Grigoryan, I. G. 15  
 Grill, V. 85, 148, 168, 317, 320  
 Grilli, A. 144  
 Grimaldi, S. 1133  
 Grimm, M. 799  
 Grimmichova, T. 726  
 Gris, F. 1033  
 Grishchenko, M. 746  
 Groele, L. 970  
 Groen, A. K. 644  
 Groenier, K. H. 135, 155, 345, 932, 1018, 1149  
 Grøndahl, C. 177  
 Groop, L. 11, 107, 108, 270, 278, 284, 496, 530, 558, 694, 1192  
 Groop, P.-H. 36, 154, 156, 170, 188, 237, 266, 273, 327, 1135, 1137, 1176  
 Groothuis, J. 1022  
 Grosfeld, A. 414  
 Grosman, B. 193  
 Gross, J. 243, 581  
 Groth, M. 590  
 Grover, G. 889  
 Gruber, D. 411  
 Gruden, G. 137, 655, 1133, 1139  
 Gruenberger, J.-B. 784, 854, 1104  
 Grulich-Henn, J. 898  
 Grunler, J. 1213, 1214  
 Grunnet, L. G. 558  
 Gruson, D. 272  
 Grzanka, M. 333, 1026  
 Grzelak, P. 928  
 Grzeszczak, W. 657  
 Gu, H. F. 893  
 Gual, P. 20  
 Guan, B. 752  
 Guanyabens, E. 164  
 Guarino, D. 583  
 Guarino, E. 1085  
 Guarino, M. P. 554  
 Guay, C. 214, 216  
 Gudbjörnsdottir, S. 51, 54, 182  
 Gueddah, L. 330  
 Guerardel, A. 491  
 Guerci, B. 5, 781  
 Guerra, E. 676  
 Guigas, B. 611  
 Guillaume, A. 989  
 Guillen, C. 533  
 Guimond, S. 392  
 Guinness, M. 906  
 Guiot, P. 1269  
 Guja, C. 280, 342  
 Gulichsen, E. 1160  
 Gulko, T. P. 458  
 Gullberg, B. 288, 869  
 Gullestad, L. 1226  
 Gulseth, H. L. 571  
 Gumprecht, J. 657  
 Günaydin, G. 937  
 Gundgaard, J. 228, 969  
 Gunn, D. 302  
 Günther, A. L. B. 881  
 Guo, H. 338  
 Guo, H. 827  
 Guo, L. 835  
 Gupta, S. K. 851, 852  
 Gurgul-Convey, E. 100, 498  
 Gurlo, T. 538  
 Gurney, K. 798  
 Gustov, A. V. 15  
 Guthke, R. 590  
 Guthoff, R. F. 44  
 Guthridge, M. A. 534  
 Gutierrez-Repiso, C. 672  
 Guttierrez, M. J. 742  
 Guyard Boileau, B. 1067  
 Guzman, J. 5, 781  
 Guzman-Perez, V. 550  
 Guzyk, M. 1207  
 Gylfe, E. 68, 102  
 Gylling, H. 722  
 Gyntelberg, F. 978  
 Gysemans, C. 446

## H

- Ha Van, G. 1167  
 Haahr, H. L. 909, 912, 923, 924, 925  
 Haak, T. 992, 1032  
 Haas, K. 664  
 Haastert, B. 1006, 1007  
 Haatanen, N. 240  
 Habib, A. M. 576  
 Hach, T. 770  
 Hada, Y. 734  
 Hadjadj, S. 80, 153, 1122  
 Hadour, G. 812  
 Haering, H.-U. 542, 683, 685  
 Hafez, M. 1025  
 Hafko, R. 438  
 Hagen, B. 1024  
 Hagner, N. 863  
 Hagopian, W. 146, 452  
 Haidar, A. 195  
 Haider, A. 888  
 Hainer, V. 726  
 Hajek, M. 74  
 Halama, A. 670  
 Halban, P. A. 139, 259, 389, 402, 407, 546  
 Hale, L. 1125  
 Hale, P. 1  
 Half, G. 711  
 Halkova, T. 726  
 Hall, E. 105, 142  
 Hallahan, N. 597  
 Hallahan, N. J. 649  
 Halmai, R. 1144  
 Hamada, I. 858  
 Hamamoto, Y. 733  
 Hamari, S. 473



- Hamasaki, A. 179, 363  
 Hamdouch, C. 547  
 Hamidi Shishavan, M. 1126  
 Hamman, R. F. 1238  
 Hammersley, S. 358, 463  
 Hammes, H.-P. 189, 1119  
 Hampe, C. S. 480  
 Han, C. 1075  
 Han, H.-J. 891  
 Han, J. 365  
 Han, J. 799, 800, 804, 832  
 Han, N. 509, 889, 1151  
 Han, S. 890  
 Han, W. 30  
 Hanafusa, T. 783  
 Hanaire, H. 1067  
 Hanamoto, T. 589  
 Hanatani, S. 661  
 Hancock, M. R. 984  
 Handelsman, Y. 39, 920  
 Handgraaf, S. 566  
 Handlos, L. N. 370, 377  
 Handsaker, J. C. 1178  
 Haneda, M. 578, 741, 1101  
 Hanefeld, M. 199, 231  
 Hanna, S. 456  
 Hannukainen, J. C. 8, 565, 601, 1261  
 Hansen, C. 114  
 Hansen, D. L. 10, 588, 1245  
 Hansen, H. S. 824  
 Hansen, K. B. 824  
 Hansen, L. 238, 275, 276  
 Hansen, M. 132  
 Hansen, R. J. 42  
 Hansen, T. 297, 334, 1083  
 Hansen, T. K. 13, 1130, 1195  
 Hansen, T. S. 1159  
 Hansikova, J. 665  
 Hanson, M. E. 1236  
 Hanssen, K. F. 1162  
 Hanssen, N. 133  
 Hansson, O. 107, 558  
 Hanzelka, K. 498  
 Hanzu, F. A. 645  
 Hara, K. 305, 734  
 Hara, S. 313, 866  
 Harada, N. 179, 363  
 Harada, N. 446  
 Harada, T. 179  
 Harada, T. 779  
 Harcourt, B. E. 151  
 Hardin, D. S. 201  
 Harding, E. 1021  
 Hardman, M. 1184  
 Hardy, A. 1017  
 Hardy, E. 744, 754, 758  
 Hardy, T. A. 351  
 Hare, K. J. 573  
 Harikrishnan, V. 704, 1229, 1231  
 Häring, H.-U. 299, 382, 631  
 Harjutsalo, V. 156, 170, 237, 1137  
 Härkönen, T. 273  
 Harman, D. J. 1216  
 Harnois, T. 1122  
 Harris, A. 774, 776  
 Harris, R. 1216  
 Harris, S. B. 228, 926, 944  
 Harrison, L. 794  
 Harrison, L. C. 424  
 Harsunen, M. H. 325  
 Hartemann, A. 1091  
 Hartmann, A. 149  
 Hartwig, S. 654, 679  
 Harvey, R. 1060  
 Hasegawa, G. 258, 568  
 Hasegawa, H. 648  
 Hashimoto, N. 785  
 Hashimoto, N. 816  
 Hashinaga, T. 257  
 Hassan, Z. 381  
 Hassan, Z. 681  
 Hasslacher, C. 1039  
 Hastrup-Nielsen, H. 912, 924  
 Hata, M. 1107, 1108  
 Hatlapatka, K. 399, 413  
 Hattersley, A. T. 53, 332, 358, 371, 463  
 Hattori, H. 894  
 Hatzis, G. 1129  
 Hauert, C.-A. 1190  
 Haug, C. 1043  
 Hauge-Evans, A. C. 385  
 Haukka, J. 616  
 Hauner, H. 287, 717  
 Haurigot, V. 191  
 Hauser, R. 906  
 Hautakangas, M.-R. 240  
 Havrdova, T. 435, 1152  
 Hawkins, N. 746  
 Hawthorne, W. J. 424  
 Hayashi, T. 1197  
 Hayato, F. 172  
 Hayward, A. 1209  
 Hazart, J. 1003  
 He, A. 752  
 He, B. 170  
 He, C. 283  
 He, J. 365, 994  
 He, P. 1124, 1212  
 He, S. 368  
 He, W.-Y. 312, 375  
 He, X. 1183  
 He, Y.-L. 843  
 Healy, G. M. 79  
 Heaton, N. 441  
 Hebda-Szydło, A. 1068  
 Hécart, A.-C. 730  
 Heckermann, S. 961  
 Hedenbro, J. 11  
 Hegele, R. A. 174  
 Heianza, Y. 313, 866, 1217, 1243  
 Heier, M. 1161  
 Heiker, J. 499  
 Heilig, H. G. H. J. 65, 644  
 Heim, K. 103, 294  
 Heinemann, L. 194, 196  
 Heinke, P. 424, 1047  
 Heinonen, I. 601  
 Heinrich, J. 895  
 Heinrichs, M. 737  
 Heise, T. 909, 912, 913, 914, 1045  
 Heller, S. R. 2, 225  
 Heller, T. 1098  
 Hellstrand, S. 869  
 Hellstrom, P. 47  
 Helminen, O. 240  
 Helquist, P. 502  
 Hempel, A. 98  
 Hempler, N. F. 960  
 Henderson, J. 341  
 Henkel, E. 231  
 Hennige, A. M. 685  
 Henning, R. H. 1126  
 Henriksen, J. 114  
 Henriksen, K. 114  
 Henry, R. R. 109, 244, 1042  
 Herbach, N. 505  
 Herder, C. 103, 294, 316, 559  
 Herman, W. H. 201  
 Hermanns, N. 251, 974, 992, 1032  
 Hermanova, H. 298  
 Hermans, M. P. 272, 1268  
 Hermansen, K. 296, 666  
 Hernández, C. 1112, 1117, 1267  
 Hernandez, E. 1076  
 Hernández, M. 328  
 Herold, K. C. 147, 452  
 Herpertz, S. 974  
 Herranz de la Morena, L. 84  
 Herring, R. 995  
 Herrmann, J. L. 1225  
 Herrmann, K. 832  
 Hertel, N. T. 276  
 Hertle, E. 1239  
 Herzfeld de Wiza, D. 92, 543, 654  
 Hesney, M. 757  
 Hess, G. 855  
 Hesse, B. 262  
 Hidas, A. 1248  
 Hietala, K. 188  
 Hijmans, A. 659  
 Hildebrandt, T. 398  
 Hilding, A. 893  
 Hilgers, R. D. 1006, 1007  
 Hill, M. 74, 75, 726  
 Himmelbauer, H. 27  
 Hindy, G. 290  
 Hine, D. L. 302  
 Hirano, T. 778, 786  
 Hiromine, Y. 650  
 Hirose, H. 301  
 Hirose, N. 885  
 Hirose, T. 865  
 Hirota, D. 62, 1115  
 Hirota, Y. 816  
 Hirsch, I. B. 41  
 Hirst, J. A. 788  
 Hirvonen, J. 28  
 Hisada, M. 839  
 Hisadome, C. 779  
 Hisadome, K. 716  
 Hivert, M.-F. 82  
 Hjelmæth, J. 29  
 Hoang, T. T. V. 625  
 Hoashi, S. 1064  
 Hoche, B. 35  
 Hodeck, K. 997  
 Hodgson, T. S. 922  
 Hoebaus, C. 134  
 Hoehn, K. L. 591  
 Hoekstra, J. B. L. 65, 184, 644, 677  
 Hoene, M. 631  
 Hoey, H. M. C. 963, 1015  
 Hoffmann, S. 189  
 Hofmeister-Brix, A. 390  
 Højlund, K. 223, 271, 594  
 Holewa, D. D. 593  
 Holkova, K. 88  
 Holl, R. W. 307, 314, 322, 897, 898, 964  
 Hollander, P. A. 117, 910, 911, 939  
 Holleman, F. 65, 644, 677  
 Höller, E. 453  
 Hollister-Lock, J. 175  
 Holmager, P. 978  
 Holman, N. 203  
 Holscher, C. 579  
 Hölscher, E. 997  
 Holst, J. J. 10, 246, 248, 296, 334, 409, 572, 573, 582, 588, 644, 666, 713, 824  
 Holstein, P. 253, 1164  
 Home, P. D. 3, 951  
 Homem, F. 1225  
 Homma, H. 1100  
 Hompesch, M. 742, 747, 908, 925  
 Hong, Y. 747  
 Honjo, J. 1101, 578  
 Honjo, S. 733  
 Honka, H. 1261  
 Hopkins, H. 995  
 Hopkinson, H. E. 225  
 Horan, J. 1263  
 Hori, W. 111  
 Horikawa, C. 313, 866, 1243  
 Horikawa, Y. 640  
 Hornemann, S. 260  
 Horowitz, M. 247, 820  
 Horsch, M. 670  
 Hoshian, F. 885  
 Hoshina, S. 165  
 Hosokawa, M. 648, 708  
 Hoti, F. 616  
 Hougaard, P. 238  
 Houweling, S. T. 1018  
 Hövelmann, U. 912  
 Hovendahl, C. 248  
 Hovendal, C. 582  
 Hovorka, R. 194, 195  
 Howey, D. C. 913, 916, 917  
 Hoyem, P. 13, 1195  
 Hrabé de Angelis, M. 299, 635, 670  
 Hsieh, S. Dong. 313, 866  
 Hu, J. 1155  
 Hu, J. 175  
 Hu, L. 368  
 Hu, Y. 392  
 Huang, G. C. 378, 381, 441  
 Huang, S. 632, 817  
 Huang, T. 746  
 Huang, W. 825  
 Huang, Z. 1183  
 Hübschle, T. 809, 811  
 Huda, M. S. B. 359  
 Hudson, M. 463  
 Hugtenburg, J. G. 354, 1002  
 Huh, K.-B. 1008, 1242  
 Huijberts, M. 133, 1255  
 Hummel, S. 325  
 Humpert, P. M. 154, 1135  
 Humphreys, W. G. 749  
 Humphriss, E. 821  
 Hunt, K. F. 359, 728  
 Hunt, S. C. 587  
 Hurley, L. 1188  
 Hursting, S. D. 235  
 Hurtado-Carneiro, V. 552  
 Husa, J. 671  
 Huskinson, A. C. 613  
 Hussain, A. 975  
 Hutton, J. C. 267  
 Hvidoere Study Group 963, 1015  
 Hvolris, L. 1245  
 Hwang, J.-H. 310  
 Hwang, O.-K. 891  
 Hynes, L. 999  
 Hyo, T. 219, 885  
 IA3 Investigators 1013  
 I  
 Iaconelli, A. 584  
 Iacopi, E. 1186  
 Iakoubov, R. 220

- Iamandi, C. 1269  
 Iaquinto, M. 495  
 Ibañez, B. 166  
 Ibata, J. 1147  
 Ibrahim, H. 875  
 ICCD Study Group 1017  
 Ichikawa, M. 698  
 Icks, A. 347  
 Ide, T. 111  
 Ide, T. 376  
 Idevall-Hagren, O. 69  
 Igarashi, K. 143  
 Igata, M. 661  
 Iggman, D. 78  
 Ignell, C. 1074  
 Iida, K. T. 1243  
 Iizuka, K. 640  
 Iizuka, Y. 734  
 IJzerman, R. G. 167, 687  
 Ikeda, H. 733  
 Ikeda, S. 767, 768  
 Ikeda, T. 589, 927  
 Ikegami, H. 171, 650  
 Ikonomidis, I. 1240  
 Ikramuddin, S. 585  
 Ikuyama, S. 698  
 Ilias, A. 428, 714  
 Iliou, M.-C. 1224  
 Ilkavets, I. 707  
 Illig, T. 103, 294  
 Iloeje, U. H. 755  
 Ilonen, J. 240, 265, 266, 274, 277, 326, 473  
 Ilyin, A. V. 465  
 Imagawa, A. 282, 423, 469  
 Imagawa, M. 698  
 Imai, J. 143  
 Imamoglu, S. 996  
 Imamura, M. 301  
 Immonen, H. 8, 565  
 Ina, K. 1197  
 Inagaki, N. 179, 363, 648, 708, 718, 847  
 Incalza, M. 125, 1260  
 Ingelgård, A. 777  
 Ingwersen, S. H. 460, 805  
 Inoue, H. 506  
 INTERPRET Study Group 1058  
 Ioacara, S. 342  
 Ionescu-Tirgoviste, C. 280, 342  
 Iosup, A. 1265  
 Ippoliti, A. 1191  
 Iqbal, N. 750  
 Irgens, H. U. 625  
 Irminger, J.-C. 139  
 Irmiler, M. 635  
 Irvine, S. 358  
 Isajeva, D. 1208  
 Isajevs, S. 1208  
 Ishibashi, R. 1124, 1212  
 Ishibashi, S. 319  
 Ishigaki, Y. 143  
 Ishii, H. 831  
 Ishikawa, T. 1212  
 Ishizawa, K. 894  
 Ishizu, T. 1217  
 Ishizuka, T. 589, 927  
 Isken, F. 712  
 Ismail, K. 359, 958  
 Isomaa, B. 482  
 Itariu, B. K. 880  
 Itaya-Hironaka, A. 525  
 Itoh, H. 1197  
 Itoh, H. 462  
 Ivanyi, T. 202  
 Ivarsson, S. A. 278  
 Iwababu, M. 113  
 Iwahashi, H. 282, 423, 469  
 Iwai, R. 785  
 Iwasaki, H. 1217  
 Iwasaki, M. 885  
 Iwasaki, N. 335, 337  
 Iwata, M. 301  
 Izarra, A. 419  
 Izumida, Y. 734  
**J**  
 J. Doyle III, F. 1060  
 Jabbour, A. D. 1028  
 Jabbour, S. 758  
 Jaber, Y. 1094  
 Jaber, Y. 32, 116  
 Jackson, M. 906  
 Jacober, S. J. 24, 914, 916, 917, 918, 919  
 Jacobsen, L. 805  
 Jacobsen, S. 297  
 Jacobsen, S. H. 10, 588  
 Jacovetti, C. 211, 216  
 Jacqueminet, S. 1091  
 Jaen, J. C. 31  
 Jahan, F. A. 681  
 Jahan, K. 1078  
 Jähnert, M. 597  
 Jaidane, A. 875  
 Jakobsen, P. 1160  
 Jakobsson, I. 69  
 Jakubowicz, D. 567  
 Jallon, P. 1033  
 James, C. 613  
 James, D. E. 91  
 Jan, A. 393  
 Janas, I. 1068  
 Jáñez Furio, M. 84  
 Jang, H. C. 852  
 Janovska, P. 665  
 Jansà, M. 479  
 Jansen, H. 868  
 Jansen, H. J. 66, 942  
 Janssen, P. 162  
 Jansson, J.-O. 1213  
 Japan CDM Investigators Group 1197  
 Jarek-Martynowa, I. 1148  
 Jarosz-Chobot, P. 1058  
 Jauch, J. 459  
 Jauhainen, M. 327  
 Jaume, D. 605  
 Javorsky, M. 298  
 Jayawardena, R. 1097  
 Jazet, I. M. 611, 731  
 Jeandidier, N. 23  
 Jebunnesa, F. 1078  
 Jedinakova, T. 435  
 Jeffcoate, W. J. 1168  
 Jeitler, M. 671  
 Jelenik, T. 22, 129, 131  
 Jelsing, J. 769  
 Jendle, J. 957  
 Jenkins, A. 1054  
 Jensen, C. B. 2, 709, 826  
 Jensen, K. L. 262, 442, 481  
 Jensen, R. 634  
 Jensen-Urstad, K. 1189  
 Jenssen, T. G. 149  
 Jenum, A. K. 882, 1071  
 Jeon, H.-J. 1127  
 Jeon, J. 890  
 Jeon, W.-S. 372, 1131  
 Jeppesen, A. B. 218  
 Jeppesen, C. B. 37, 915  
 Jepson, B. 147  
 Jesus, I. 58, 59  
 Jett, D. 547  
 Ji, L. 349, 795, 835, 836, 948, 977  
 Ji, Q. 948  
 Jia, J. 480  
 Jia, W. 73, 349, 835, 872  
 Jiang, H. H. 201, 202  
 Jiang, Y. 1009  
 Jiletcovici, A. O. 940  
 Jimenez, A. 645  
 Jimenez, J. G. 1236  
 Jimenez, S. 1141  
 Jimenez, V. 418, 444, 448  
 Jin, H. 1145  
 Jin, Y. 489  
 Jin, Z. 489  
 Jinga, M. 877  
 Jirkovská, A. 255, 256  
 Jiun-Shen, B. 1184  
 Jo, K. 1111  
 Joensen, L. E. 960, 976  
 Joergensen, C. 1132, 1146, 1244  
 Johannesen, J. 614  
 Johansen, A. 614  
 Johansen, O. E. 148, 1226, 1233  
 Johansen, T. 38, 39, 117  
 Johansson, G. 690  
 Johansson, H. 519  
 Johansson, H.-E. 78  
 Johansson, L. 78, 748, 751  
 Johansson, L. A. 261  
 Johansson, S. 625  
 Johne, C. 395, 595  
 Johns, N. 1090  
 Johnson, J. A. 52, 329, 350  
 Johnson, J. D. 67, 534, 536  
 Johnson, K. 457  
 Johnson, S. 146  
 Johnson, S. 581  
 Johnson, S. T. 350  
 Johnson, T. 172  
 Johnson-Levonas, A. O. 827, 1235  
 Johnsson, K. 242, 743  
 Johnston, P. 946  
 Jones, A. G. 332, 371  
 Jones, J. G. 610  
 Jones, K. L. 247  
 Jones, N. 1202  
 Jones, P. M. 378, 379, 381, 385, 396, 432, 440  
 Jones, R. B. 771  
 Jonker, L. 65, 644  
 Joo, E. 179  
 Joost, H.-G. 27, 673  
 Joosten, L. 263  
 Joosten, L. A. B. 659  
 Jordan, J. 697  
 Jørgensen, J. V. 238  
 Jørgensen, M. E. 700  
 Jørgensen, N. B. 10, 588  
 Jørgensen, S. M. 713  
 Jørgensen, S. W. 96  
 Jørnås, A. 304, 446, 514  
 Jørum, E. 1162  
 Jose, B. 1270  
 Joseph, G. 704, 1229, 1231  
 Joslowski, G. 316, 325, 881  
 Jothydev, S. 360  
 Jotic, A. 180, 475  
 Joubert, M. 989  
 Jovanovic, L. 1060  
 Jozkowicz, A. 254  
 Juhl, C. B. 223  
 Jung, H.-S. 509, 889  
 Juntti-Berggren, L. 483  
 Juretschke, H.-P. 809  
 Juul, E. 377  
**K**  
 Kaňková, K. 152  
 Kabir, F. 681  
 Kacem, M. 330  
 Käck, C. 442  
 Kaddai, V. 389  
 Kadoglou, N. 1240  
 Kadono, M. 258  
 Kadowaki, T. 113, 172, 305, 460, 734  
 Kagimura, T. 847  
 Kahl, S. 310  
 Kahl, S. D. 120  
 Kahle, M. 635  
 Kahleova, H. 74, 75  
 Kahn, C. Ronald. 590  
 Kahn, M. 19  
 Kaiser, N. 504  
 Kajbaf, F. 790  
 Kajita, K. 589, 927  
 Kajitani, N. 62, 1115  
 Kajiwara, T. 1100  
 Kakei, M. 405, 488  
 Käkälä, P. 722  
 Kaku, K. 70, 301  
 Kalil, J. 404  
 Kalko, S. G. 684  
 Kalliokoski, K. K. 601  
 Kalopita, S. 1109  
 Kaltenboeck, R. 134  
 Kalvinsh, I. 1208  
 Kalynska, L. N. 458  
 Kamada, T. 779  
 Kamaratos, A. 56  
 Kamath, S. 711, 791  
 Kamikubo, T. 779  
 Kaminska, D. 722  
 Kamiya, H. 1107, 1108, 1247  
 Kampmann, U. 13  
 Kan, C. 958  
 Kaneko, S. 636  
 Kang, E.-K. 891  
 Kankova, K. 1128  
 Kannard, B. 1059  
 Kanno, A. 533  
 Kanno, S. 1101  
 Kanzaki, M. 389  
 Kanzleiter, T. 597, 673  
 Kapanen, J. 601  
 Kapantais, E. 1082  
 Kapellen, T. M. 314, 898  
 Kapitza, C. 813, 814, 826  
 Kaplan, D. D. 587  
 Karagiannis, T. 241  
 Karakosta, P. 86  
 Karalliedde, J. 793  
 Karamanos, B. 1070  
 Karamoutsios, A. 477  
 Karbowska, S. 227  
 Karime, B. 1162  
 Karlsson, H. K. 28  
 Karmi, A. 565  
 Karpe, F. 629  
 Karsdal, M. A. 114

- Karstoft, K. 600  
 Kashiwagi, A. 301, 739  
 Kasichayanula, S. 747, 749, 757  
 Kassanos, D. 1081  
 Kastenmüller, G. 87, 108, 635, 670  
 Katagiri, H. 143  
 Kataoka, H. 62  
 Katchunga, P. B. 1268  
 Katebe, M. A. 1123  
 Kato, K. 1247  
 Kato, T. 822  
 Kato, Y. 1247  
 Katsilambros, N. 366, 724, 867, 1109, 1129, 1241  
 Katsura, M. 70  
 Katsuta, H. 175  
 Katulanda, P. 1097  
 Katz, A. 329  
 Kaufman, F. R. 193, 627, 1042, 1059  
 Kaufman, J. M. 654  
 Kaufman, K. D. 827, 834  
 Kauschke, S. 398  
 Kautzky-Willer, A. 1066  
 Kawabata, Y. 171  
 Kawaguchi, M. 243  
 Kawahara, M. 503, 1257  
 Kawai, S. 318  
 Kawakami, M. 430, 488  
 Kawamori, D. 175  
 Kawamori, E. 301  
 Kawamoto, E. 973  
 Kawamura, H. 1124, 1212  
 Kawasaki, E. 171  
 Kawasaki, S. 661  
 Kawasaki, Y. 733  
 Kawazu, S. 376  
 Kay, T. W. 531  
 Kaye, P. 1216  
 Kazda, C. M. 112  
 Kazdova, L. 551  
 Kazuo, H. 172  
 Kazuta, K. 739  
 Keane, F. 1001  
 Keck, M. 414  
 Keenan, B. 193  
 Kefalogiannis, N. 1157  
 Keigwin-Harris, R. A. 1142  
 Keipert, S. 26, 130  
 Keller, C. 460  
 Keller, M. 694  
 Kellner, C. 998  
 Kelly, L. 1188  
 Kelly, R. P. 112, 818  
 Kelstrup, L. 1083  
 Kempf, K. 1006, 1007  
 Kempler, P. 1158  
 Kenward, M. G. 265, 326  
 Kerenyi, Z. 1092  
 Kerr, P. G. 1105  
 Kerr-Conte, J. 434, 467, 522  
 Kersten, S. 598  
 Kesavadev, J. 360  
 Kesikli, A. S. 937  
 Kessler, B. 505  
 Kewelow, W. C. 952, 959  
 Keyes-Elstein, L. 454  
 Keyhani Nejad, F. 712  
 Khalil, S. 79, 1064  
 Khan, I. 681  
 Kharitonov, A. 25  
 Khaw, K.-T. 173  
 Khoo, J. 1187  
 Khunti, K. 228, 311, 621  
 Khutoryansky, N. 806  
 Kido, Y. 506, 533  
 Kiec-Wilk, B. 333  
 Kieffer, T. J. 420, 443, 570, 578  
 Kiess, W. 678, 499  
 Kikuchi, M. 376  
 Kildegaard, J. 915  
 Kiljanski, J. 5, 781, 940  
 Kim, B.-J. 1250  
 Kim, C.-S. 1111  
 Kim, C.-H. 355  
 Kim, D.-J. 1143  
 Kim, D. 890  
 Kim, D. M. 851  
 Kim, E.-H. 355  
 Kim, H.-K. 355  
 Kim, H. 890  
 Kim, J. 1111  
 Kim, J.-Y. 1250  
 Kim, J. A. 851, 852  
 Kim, J. S. 1111  
 Kim, M. 509, 889, 1151  
 Kim, M.-K. 891  
 Kim, S. 372  
 Kim, S. 509, 889, 1151  
 Kim, S. 891  
 Kim, S. W. 852  
 Kim, T. 1151  
 Kim, T. 1151  
 Kim, T. 1177  
 Kim, T. 509, 509  
 Kim, T. 889  
 Kim, T. 889  
 Kim, W. Y. 13  
 Kim, Y. S. 851  
 Kimura, H. 525  
 Kimura, H. 718  
 Kimura, T. 401  
 Kimura-Koyanagi, M. 533  
 King, A. J. F. 378, 440  
 King, K. L. 146  
 King, R. 613  
 Kingo, L. 988  
 Kinmonth, A.-L. 183  
 Kinote, A. P. 652  
 Kinsley, B. 364  
 Kipnes, M. S. 821  
 Kipp, B. 1021  
 Kirakossian, D. 425  
 Kircher, R. 930, 1055  
 Kirlaki, E. 1157  
 Kirveskari, J. 327  
 Kirveskoski, T. 473  
 Kirwan, B. 79, 1064, 1069  
 Kiss, I. 923  
 Kissner, T. 737, 812  
 Kistorp, C. 138, 978  
 Kitada, Y. 927  
 Kitamoto, Y. 219  
 Kitamura, T. 589  
 Kitamura, T. 686  
 Kitamura, Y. 111  
 Kitanoya, H. 421  
 Kitatani, N. 885  
 Kitsunai, H. 578  
 Kivimäki, M. 356, 357  
 Kiviranta, R. 8, 565  
 Kjær, A. 262  
 Kjeldsen, T. B. 37, 915  
 Kjøbsted, R. 217  
 Klaff, L. 1040  
 Klaus, S. 26, 130  
 Klee, P. 491  
 Kleefstra, N. 135, 155, 345, 1018, 1149  
 Kleefstra, N. 932  
 Klein, D. 1, 805  
 Klein, K. 618  
 Klein, M. 167  
 Klein, T. 35, 769, 771  
 Kletzien, R. F. 593  
 Klimcakova, L. 298  
 Klimontov, V. V. 1134  
 Klingspor, M. 664  
 Klinglmueller, F. 671  
 Klonoff, D. C. 119, 627, 907  
 Kloos, C. 952, 959, 1098, 1205  
 Klötting, I. 481  
 Klötzer, H.-M. 1043  
 Kluge, R. 673  
 Klupa, t. 333, 1026  
 Klüppelholz, B. 310  
 Knebel, B. 22, 129, 445, 679  
 Knight, B. A. 371, 463  
 Kniotek, M. 236  
 Knip, M. 237, 240, 265, 266, 273, 274, 277, 326, 473  
 Knoch, K.-P. 331  
 Knop, F. 334, 644, 248, 572, 573, 582, 824  
 Knopff, A. 895  
 Knotek, M. 1150  
 Knudsen, S. H. 600  
 Knuuti, J. 601, 1261  
 Ko, K. 1143  
 Kobayashi, H. 165  
 Kobayashi, K. 1073  
 Kobayashi, K. 1124, 1212  
 Kobayashi, K. 1217  
 Kobayashi, T. 171  
 Kobayashi, Y. 1107, 1108  
 Koblik, T. 254  
 Kochanek, S. 418  
 Kocik, M. 435  
 Kockum, I. 278  
 Kocsis, G. 1058  
 Kodama, S. 313, 866, 1073, 1217, 1243  
 Koder, R. 62, 1115  
 Koehler, C. 199, 231  
 Koehler, W. 953, 1005, 1035  
 Koenen, T. B. 66  
 Koenig, S. 146  
 Koester, A. 42  
 Kogevinas, M. 86  
 Koh, G. 891  
 Köhler, B. 44  
 Köhler, G. 924  
 Köhler, S. 18  
 Kohmura, Y. 318  
 Koizumi, T. 698  
 Kojima, I. 589  
 Kokkinos, A. 1109  
 Kokkinos, A. 724, 1129, 1241  
 Kolaitis, N. 474, 477  
 Koláriková, G. 438  
 Kolatsi-Joannou, M. 1209  
 Kollman, C. 195  
 Kolonics, A. 1248  
 Kolterman, O. 798  
 Komatsu, M. 831  
 Komissarova, E. S. 941  
 Komori, T. 63, 686  
 Kon, A. 718  
 Kondo, K. 143  
 Kondo, T. 140, 161  
 Kondo, T. 661  
 Kondo, Y. 363  
 Kono, M. 894  
 Konova, E. I. 1084  
 Konstantinova, M. 902  
 Konyashin, A. 1049  
 Kootte, R. S. 65  
 Kopeczy, J. 642, 665  
 Kopp, H. P. 624, 723  
 Koppensteiner, R. 134  
 Korányi, L. 569  
 Kordum, V. A. 458  
 Korhonen, P. 616  
 Koriath, M. 694  
 Körner, A. 499, 678  
 Korol, S. 489  
 Korsatko, S. 924  
 Korsgren, O. 261  
 Kosek, E. 48  
 Koshiyama, H. 733  
 Koskinen, P. 1137  
 Kosobyan, E. 1148  
 Kostareva, A. 707  
 Kostenis, E. 685  
 Kostense, P. J. 1002  
 Kostev, K. 784, 854  
 Kostic, V. S. 180  
 Kostoula, M. 1001  
 Kosutic, G. 47  
 Köthe, L. D. 246  
 Kothiwale, S. V. 1079  
 Kothiwale, V. A. 1079  
 Kothny, W. 856, 857, 859, 860  
 Kotova, O. 530  
 Kotzka, J. 22, 129, 445, 679  
 Kou, K. 462  
 Koukoulis, C. 1240  
 Kousathana, F. 1240  
 Kovacs, P. 499, 680, 694  
 Kovalenko, A. 1173  
 Kovaleva, Y. A. 792  
 Kovzun, O. I. 458  
 Kowall, B. 308, 1161  
 Kozarich, J. 822  
 Kozarzewski, M. 377  
 Kozawa, J. 423  
 Kozek, E. 1026  
 Kozlovski, P. 857, 859, 860  
 Kretowski, A. 689  
 Kraegen, E. W. 591  
 Kraft, G. 224  
 Kralli, A. 127  
 Kramar, R. 1138  
 Kramer, A. 260  
 Krämer, U. 307  
 Kramna, L. 88  
 Krasinski, A. 31  
 Kratzsch, J. 499  
 Kraunsøe, R. 132  
 Krebs, S. 505  
 Kreppel, F. 418  
 Kretowski, A. 1086  
 Kretschmann, J. 1024  
 Krippeit-Dreows, P. 382  
 Krishnan, G. 360  
 Krishnan, R. 264  
 Kristensen, J. M. 217  
 Kristensen, M. M. 599  
 Kroehl, M. 268  
 Krohn, K. 694  
 Krol, S. 437  
 Kronfeld, K. 974  
 Krumsiek, J. 87, 169  
 Krupp, D. 881  
 Kruse, J. 974  
 Kruse, M. 260, 712  
 Krusová, D. 152, 1128



- Krust, A. 566  
 Kuchmerovska, T. 1207  
 Kudlova, P. 879  
 Kudva, Y. 1056  
 Kuestermann, E. 501  
 Kuhlmann, O. 767  
 Kuhlow, D. 590  
 Kuka, J. 1256  
 Kulkarni, M. 1172  
 Kulkarni, R. 175  
 Kullberg, J. 78  
 Kulmatycki, K. 843  
 Kulozik, F. 1039  
 Kulzer, B. 992, 1032  
 Kulzer, B. 974  
 Kumar, A. 1079  
 Kumar, S. 726, 1082  
 Kumar, S. 732  
 Kumareswaran, K. 195  
 Kun, A. 1092  
 Künnecke, W. 1045  
 Kuraeva, T. 623  
 Kurano, M. 779  
 Kurashina, T. 405  
 Kuricová, K. 152, 1128  
 Kurihara, S. 171  
 Kurisu, S. 1147  
 Kurita, K. 1108  
 Kuroda, Y. 469  
 Kurome, M. 505  
 Kurose, T. 219, 885  
 Kus, V. 665  
 Kusinska, K. 254  
 Kutscherauer, G. 736  
 Kuulasmaa, T. 722  
 Kuwata, H. 219, 885  
 Kuzmicki, M. 1086  
 Kuzuya, T. 783  
 Kvapil, M. 947  
 Kwon, M. 509, 889, 1151  
 Kwon, M. 889  
 Kyrou, I. 726
- L**
- L'hoste, S. 414  
 La Rosa, S. 495, 711  
 Laakso, M. 284, 722  
 Labarbuta, R. 513, 1260  
 Labriola, L. 404  
 Labudzynski, D. 450  
 Lacaria, E. 1072, 1093  
 Lacatusu, A. 900, 903  
 Lacić, I. 438  
 LaCreta, F. P. 747, 749, 757  
 Lacroix, M. 82  
 Lacy, P. 1146, 1244  
 Ländin, M. 97  
 LAF237A1308 Study Group 858  
 Lage, R. 418, 448  
 Lagrée, V. 397  
 Lahera, L. 1141  
 Laine, A. P. 274, 277  
 Lajer, M. S. 1130, 1132, 1146, 1244  
 Lajoix, A. 397  
 Lakdawalla, D. 619  
 Lakey, J. R. T. 264, 439  
 Lakey, W. 204  
 Lalau, J.-D. 790  
 Lalic, K. 180, 475  
 Lalic, N. M. 180, 475, 1058, 1070  
 Laloum, J. 484  
 Lam, A. W. Y. 443  
 Lam, E. C. Q. 922  
 Lam, K. S. L. 1031  
 Lama, C. 1141  
 Lamanna, C. 343  
 Lamb, H. J. 1219  
 Lamb, M. 264, 439  
 Lambadiari, V. A. 1240  
 Lambers Heerspink, H. J. 770  
 Lambert, I. H. 218  
 Lambert-Porcheron, S. 209  
 Lamberts, E. J. F. 354  
 Lambris, J. D. 158  
 Lammertsma, A. A. 1219  
 Lamotte, M. 956  
 Lamounier, R. N. 604  
 Lamprinou, A. 1081  
 Lamri, A. 289, 300  
 Landau, Z. 119, 1000  
 Landgraf, K. 678  
 Landgraf, W. 199, 947  
 Landheer, S. 1126  
 Landi, A. 137  
 Ländin, M. 207  
 Landstedt-Hallin, L. 796  
 Landwehr, S. 103, 294  
 Lang, G. E. 192  
 Lang, J. 397, 484  
 Langdahl, B. L. 296  
 Lange, K. 962, 964, 1015  
 Langenberg, C. 173  
 Langkilde, A. M. 748, 750, 751, 777  
 Langkramer, S. 255  
 Langlois, M.-F. 350  
 Lantieri, O. 289, 300  
 Lao, G. 1116  
 Lapauw, B. 654  
 Lapolla, A. 1070, 1085  
 Laptev, D. 623  
 Lapuerta, P. 772, 775  
 Lara-Lemus, R. 338  
 Larger, E. 80  
 Larizza, D. 336, 1175  
 Larsen, A. 223  
 Larsen, J. 911  
 Lasaite, L. 965  
 Lasheras, J. 1193  
 Lashinger, L. M. 235  
 Lassenius, M. I. S. 327  
 Lassota, N. 617, 909, 921, 926  
 Lathouris, P. 369  
 Lathrop, M. 553  
 Latif, Z. A. 951  
 Lau, D. 329  
 Laub, M. 1264  
 Laubner, K. 507, 1081  
 Laue, S. 499  
 Lauffer, L. M. 220  
 Laugen, J. 585  
 Laugesen, E. 13  
 Laumen, H. 287  
 Lauria, A. 115  
 Laurila, E. 558  
 Lauro, D. 1191  
 Lauro, R. 64, 159, 160, 643, 1191  
 Lavielle, M. 209, 306  
 Laviola, L. 513, 675, 125, 1139, 1260  
 Lawrence, J. M. 353  
 Lawrence, R. T. 591  
 Lawson, S. 373  
 Lawton, A. 373  
 Laybutt, D. R. 515  
 Laybutt, R. 216  
 Lázár, M. 1158  
 Le Berre, J.-P. 1266  
 Le Floch, J.-P. 1106  
 Le Gall, M. 414  
 le Roux, C. W. 9  
 Le Thi, T. D. 709  
 Le Tourneau, T. 553  
 Lebailly, B. 283  
 Lebreton, F. 484  
 Lecadet, J. 1003  
 Lecube, A. 1267  
 Ledda, A. 324  
 Lee, D. 891  
 Lee, D. V. 541  
 Lee, E.-J. 1008, 1242  
 Lee, E. 509, 1151  
 Lee, E. 889  
 Lee, J. 1177  
 Lee, J.-S. 509, 889  
 Lee, K.-W. 890  
 Lee, L. 424  
 Lee, M. 398  
 Lee, M. 890  
 Lee, M. K. 852  
 Lee, S. 509, 889, 1151  
 Lee, S. 891  
 Lee, S. W. 1042  
 Lee, W.-Y. 372, 1131  
 Lee, Y.-H. 1127  
 Lee, Y. 1143  
 Lee, Y. M. 1111  
 Leelarathna, L. 194, 195  
 Leenen, P. J. M. 656  
 Lefebvre, B. 391, 520  
 Lehmann, R. 542, 631  
 Lehmann, T. 952, 959  
 Lehner, Z. 411  
 Lehr, S. 543, 654, 679  
 Lehtiö, J. 519  
 Lehto, M. 156, 188, 327  
 Lei, C. 365  
 Leiter, L. 350, 581, 745, 756, 763  
 Leitner, L. 880  
 Leivestad, T. 149  
 Lekakis, J. 1240  
 Lemeille, S. 259  
 Lemmens, L. C. 1022  
 Lempainen, J. 169, 277  
 Leña, M. 870  
 Lencioni, C. 1093  
 Lender, D. 119  
 Leng, Y. 632, 817  
 Lengyel, C. 1158  
 Lenoble, P. 1269  
 Lenzen, S. 100, 215, 304, 390, 446, 498, 514, 524  
 Leo, A. 1221  
 Leon, X. 418  
 Leonardini, A. 513, 125, 1260  
 Leone, A. 545  
 Leonetti, F. 874  
 Leopardi, A. 57  
 Leotta, S. 115  
 Lepomäki, V. 601  
 Lernmark, Å. 278, 481  
 LeRoith, D. 706  
 Lervang, H. 1160  
 Leslie, B. R. 747  
 Leto, G. 896  
 Leturque, A. 414  
 Leung, S. 464  
 Levin, P. 803, 986  
 Levine, J. 1056  
 Levy-Marchal, C. 321  
 Lewin, A. 822  
 Ley, L. 770  
 Li, C. 379  
 Li, F. Fei. 537  
 Li, G. 1204  
 Li, G. 403  
 Li, H. 795  
 Li, J. 102  
 Li, J. 1075  
 Li, J. 315  
 Li, J. 393, 416, 422  
 Li, J. 631  
 Li, J. 688  
 Li, L. 580, 715  
 Li, M. 977  
 Li, N. 529  
 Li, N. S. 197  
 Li, S. 1145  
 Li, S. 42  
 Li, X. 312  
 Li, X. 368  
 Li, X.-M. 375  
 Li, X.-J. 403  
 Li, Y. 1009  
 Li, Y. 1075  
 Li, Y. 1155  
 Li, Y. 1183  
 Li, Y. 4  
 Li, Y. 951  
 Li, Z. 229  
 Li Volti, G. 782  
 Liang, H. 1114  
 Liang, H. 704, 1229, 1231  
 Liao, B.-Q. M. 21  
 Liaskos, C. 724  
 Liatis, S. 366, 867  
 Lichiardopol, R. 607  
 Liepinsh, E. 1256  
 Lievens, J. 1014  
 Lieveise, L. 947  
 Liew, A. 1263  
 Liew, C. 175  
 Liguoro, D. 651  
 Likhoshapko, O. 1173  
 Liljenquist, D. 1046  
 Lillelund, C. 217  
 Lim, C. N. 112, 818  
 Lim, D.-M. 1250  
 Lim, G. E. 67, 534  
 Lim, J. 1008  
 Lima, M. J. 429  
 Lima-Rubio, F. 870  
 Limen, S. 1013  
 Lin, H.-D. 312, 375  
 Lin, J. 938, 943  
 Lin, S. 368  
 Linck, N. 72  
 Lind, K. 471  
 Lindahl, J.-P. 149  
 Lindblad, B. 278  
 Lindhout, D. 587  
 Lindqvist, A. 11  
 Lindroos, J. 671  
 Ling, C. A. 105, 107, 142  
 Ling, H. 1056  
 Ling, L. 587  
 Ling, Z. 797, 981, 986  
 Lingvay, I. 794  
 Link, M. 1043  
 Linn, T. 145  
 Linnebjerg, H. 922  
 Linnemann Jensen, M. 1102  
 Lins, P.-E. 796  
 Linz, D. 811  
 Linz, W. 811

- Liotti, A. 637  
 Lipar, K. 435  
 Lipinska, D. 1086  
 Lipka, M. 970, 1053  
 Lipowska, A. 1068  
 List, J. F. 242, 743  
 Literáti-Nagy, B. 569  
 Literáti-Nagy, Z. 1248  
 Lithovius, R. 1137  
 Liu, B. 378, 381  
 Liu, F. 872  
 Liu, H. 1116  
 Liu, H. 752  
 Liu, J. 1254  
 Liu, L. 1251  
 Liu, M. 338  
 Liu, X. 747, 749, 757  
 Liu, Y. 1075, 1254  
 Liu, Y. 1116  
 Liu, Y. 1222  
 Liviatan, L. 118  
 Livingston, E. 979  
 Livingstone, R. 310  
 Lizárraga-Mollinedo, E. 417, 528  
 Ljunggren, Ö. 748, 751  
 Llamazares, O. 833  
 Llaro Casas, M. G. 84  
 Llauroad, G. 725  
 Lledo, A. 48  
 Lloyd, A. 956  
 Lo, C. 1105  
 Loba, J. 928, 931, 934  
 Lobbens, S. 81  
 Locatelli, M. 1209  
 Lockwood, J. F. 120  
 Logan, D. 822  
 Loganathan, T. 34, 1262  
 Logtenberg, S. J. J. 932  
 Loh, M. Teng. 818  
 Loizeau, V. 902  
 Lombardo, F. L. 1203  
 Lommer Kristensen, P. 620  
 Long, D. A. 1209  
 Looker, H. C. 187  
 Loomans, C. J. M. 433  
 Lopes, A. 58, 59  
 Lopes, P. C. M. 557  
 Lopez, A. F. 534  
 López, J. 833  
 Lopez Medina, J. A. 293  
 Lopez-Bigas, N. 144  
 López-Ríos, L. 1258  
 Lorenz, M. 808  
 Lorenzati, B. 655  
 Lorenzini, F. 1067  
 Loric, S. 664  
 Lormeau, B. 1094  
 Lortz, S. 100, 524  
 Losa, S. 122  
 Louchami, K. 77  
 Loudovaris, T. 424  
 Lu, B. 1155  
 Lu, J. 349, 835, 948  
 Lu, Y. 365  
 Łuźniak, P. 17  
 Lubberink, M. 261, 1219  
 Lucariello, M. 545  
 Lucchesi, D. 1196  
 Lucci, D. 57  
 Luciano, R. 901  
 Lucidi, P. 938  
 Lucisano, G. 249  
 Ludvigsson, J. 146, 278, 1099  
 Luger, A. 411, 1066  
 Lui, M. 1105  
 Luijck, Y. M. 194, 196  
 Luk, C. T. 205, 206  
 Lukas-Croisier, C. 730  
 Lukashevich, V. 856, 857, 859, 860  
 Lukasova, P. 1194  
 Lukic, L. 180, 475  
 Lukic, M. L. 497  
 Lukowski, S. 707  
 Lumia, M. 326  
 Luna, A. 725  
 Lund, M. T. 132  
 Lund, N. 1009  
 Lund, S. 770  
 Lund-Andersen, H. 377  
 Lundby-Christensen, L. 1245  
 Lundh, M. 494  
 Lung, T. W. C. 788  
 Lungaro, J. 457  
 Luo, R. 403  
 Lupachyk, S. 1121, 1174  
 Lupoli, R. 586  
 Luque, A. 833  
 Luque, I. 833  
 Luskey, K. 109  
 Lutale, J. K. 1170  
 Lutgens, E. 157  
 Luzio, S. D. 983, 1142  
 Lybaert, P. 485  
 Lymberi, P. 1063  
 Lynch, P. 1041, 1052  
 Lynge, J. 460, 826  
 Lyssenko, V. 108, 284, 482  
**M**  
 Młynarski, W. 1237  
 Ma, H. 312  
 Ma, J. 1116  
 Ma, X. 752  
 Maalouf, N. M. 794  
 Maaroufi, A. 330  
 Mabley, J. G. 34, 1211, 1252, 1262  
 MacConell, L. 4, 798, 800, 825  
 Maccubbin, D. 1235  
 Mace, K. F. 24, 913, 922  
 Macedo, M. P. 610  
 Macek Jilkova, Z. 665  
 Macesic, M. V. 180, 475  
 Machann, J. 299  
 Machicao, F. 299  
 MacIntosh, G. 517  
 Mackowiak, L. 1057  
 MacLean, A. 1235  
 MacLeod, K. 1037, 1038  
 Madani, S. 714  
 Maddaloni, E. 115  
 Madec, A.-M. 532  
 Madeddu, P. 122, 123  
 Mader, J. K. 194, 924  
 Madsbad, S. 10, 588  
 Madsen, P. 37, 915  
 Madsen, R. 223  
 Maechler, P. 408, 523, 529  
 Maeda, S. 172, 301  
 Maedler, K. 162, 467, 499, 501, 512, 522  
 Maegawa, H. 301  
 Maffioli, P. 729, 735, 829  
 Maganaris, C. N. 1178  
 Maggini, M. 309, 1203  
 Maggs, D. 4, 618, 799, 800  
 MAGIC Investigators 284  
 Magni, L. 194  
 Magré, J. 553  
 Maguire, A. 49, 373  
 Mahadeb, Y. 272, 1268  
 Mahaffey, K. W. 764  
 Maimaitiming, S. 153  
 Maire, A. 1033  
 Maisnam, I. 873  
 Majumdar, S. R. 329  
 Mäkimattila, S. 616  
 Mäkinen, V.-P. 170, 327  
 Makino, H. 62, 1115  
 Makino, Y. 578  
 Makous, J. C. 1030  
 Makrecka, M. 1256  
 Makrilakis, K. 366, 867  
 Malaisse, W. J. 77, 485  
 Malashicheva, A. 707  
 Malecki, M. T. 254, 332, 333, 1026, 1065, 1068  
 Malenica, M. 630  
 Malgrange, D. 730  
 Malik, R. 47  
 Malinska, H. 551  
 Mallol, C. 418, 444, 448  
 Malloy, J. 798, 799, 832  
 Malpique, R. 436, 684  
 Maltezos, E. 1157  
 Maluskova, D. 1128  
 Mamdani, M. 228  
 Manabe, K. 973  
 Mañas - Martínez, A. B. 1103  
 Manco, M. 901  
 Mandal, S. 630  
 Mandøe, M. J. 824  
 Mandrup-Poulsen, T. 344, 494  
 Manes, C. N. 1157  
 Manfrini, S. 115  
 Mange, F. 20  
 Mangione, A. 76  
 Mankovsky, B. N. 1173  
 Manley, S. E. 984  
 Mann, C. J. 447  
 Mannalithara, A. 628  
 Mannic, T. 259  
 Mannucci, E. 2, 343, 1171  
 Manohar, S. 262  
 Manoharan, D. 793  
 Mansell, P. 225  
 Mansfield, T. A. 754  
 Mansinho, A. M. 987  
 Mantzur, C. 303  
 Manzoli, L. 945  
 Maqdasy, S. 1003  
 Maqueda, E. 833  
 Maran, A. 1029  
 Marando, A. 711  
 Marcelo, M. 547  
 Marchesini, G. 1192  
 Marchetti, M. 123  
 Marchetti, P. 150, 431, 437, 446, 461, 493, 511, 513  
 Marchetti, V. 64, 160  
 Marchionni, N. 343  
 Marco, A. 833  
 Marconi, J. A. 1225  
 Marcovina, S. 1238  
 Marcucci, M. 559  
 Marculescu, R. 880  
 Marcus, C. 278  
 Maresca, G. 830  
 Marette, A. 662  
 Marfia, G. A. 1180  
 Margeli, A. 1082  
 Mari, A. 108, 583, 610, 828  
 Mariano-Goulart, D. 55  
 Marini, M. 1166  
 Marino, A. 64, 159, 643  
 Marino, R. 1034  
 Mariotti, L. M. 461  
 Mariotti, R. 150  
 Mark, M. 398, 769, 771  
 Markovic, I. 475  
 Marletta, S. 418  
 Marques, N. 58, 59  
 Marques-Vidal, P.-M. 1011  
 Marra, G. 249  
 Marre, M. 3, 80, 153, 289, 300, 1070  
 Marsden, P. K. 728  
 Marselli, L. 431, 437, 446, 461, 493, 511  
 Marsh, W. J. 576, 622  
 Martelli, D. 343  
 Martelli, E. 1191  
 Martens, P. C. 573  
 Martin, A. 373  
 Martin, F. 419  
 Martin, G. 870  
 Martin, M. A. 528  
 Martin, S. 103, 294, 1006, 1007  
 Martin, S. 342  
 Martín-del-Río, R. 406  
 Martín-Núñez, G. M. 672  
 Martínez, C. 612  
 Martínez, J. P. 166  
 Martinez Calejman, C. 605  
 Martino, L. 470  
 Martinov, M. 1148  
 Martins, B. 9  
 Martins, F. O. 610  
 Marui, E. 318  
 Maruyama, T. 171  
 Mas, A. 1076  
 Mas-Gutierrez, J. A. 94  
 Masaharu, N. 786  
 Masiello, P. 437, 470  
 Masini, M. 437, 470  
 Massa, L. M. 883  
 Massaad, R. 1236  
 Massucco, P. 1163  
 Mastantuono, M. 896  
 Mastorakos, G. 1082  
 Mastrocola, R. 660  
 Mastrototaro, J. J. 193  
 Masulli, M. 945  
 Matas, Z. 1000  
 Matejko, B. 333, 1026, 1065  
 Matheson, D. 930, 1055  
 Mathiesen, E. R. 83, 1083  
 Mathieu, C. 39, 446, 615, 766, 863, 910, 911, 920  
 Matikainen, N. 853  
 Matos, L. N. 1225  
 Matouskova, J. 879  
 Matsagouras, G. 1240  
 Matsubara, K. 282  
 Matsubara, T. 1107, 1108  
 Matsuda, T. 506, 816  
 Matsui, M. 1100  
 Matsui, T. 111  
 Matsumoto, T. 885  
 Matsumura, T. 661  
 Matsuoka, A. 733  
 Matsuoka, T. 1073  
 Matsuoka, T.-A. 469  
 Matsuyama, Y. 376  
 Mattapalli, D. 843  
 Matthews, D. R. 241, 764, 1097

- Matti, P. 547  
 Mattiello, L. 232  
 Matushevskaya, E. V. 1185  
 Matveyenko, A. V. 425  
 Matz, M. 399  
 Matzur, C. 304  
 Maule, M. 324  
 Mauricio, D. 328  
 Maury, E. 876  
 Mauseth, R. 930, 1055  
 Mavilio, M. 64, 160  
 Maxhera, B. 92  
 Maxwell, M. 45  
 Mayer, G. 1138  
 Maynard, J. D. 369  
 Mayor Reyes, M. 293  
 Mayoux, E. 769, 771, 1141  
 Mazuch, J. 260  
 Mc Ternan, P. 726  
 McAllister, D. 1202  
 McAlpine, R. R. 990  
 McCallum, R. 47  
 McCann, T. W. 1057  
 McCarthy, M. I. 511  
 McCrady Spitzer, S. 1056  
 McDonald, T. J. 332, 463  
 McDonald, W. G. 593  
 McEwan, P. 956  
 McGill, J. B. 186  
 McGuire, B. 999  
 McNamara, J. 147, 452  
 McPherson, R. 1142  
 Mehana, A. E. 507  
 Mehmeti, I. 524  
 Meikle, P. J. 502, 517  
 Meinders, A. E. 611  
 Meininger, G. 243, 759, 760, 762, 763, 764, 765, 766  
 Meisinger, C. 103, 294, 308, 1161  
 Meisner, S. 248, 582  
 Meissner, T. 314, 322, 897, 898, 964  
 Meitinger, T. 103, 294  
 Melas, N. 16, 56  
 Melidonis, A. 16, 56  
 Melino, G. 643  
 Melki, V. 1067  
 Mellberg, K. 217  
 Mellett, N. 502  
 Mellgren, S. Ivar. 1162  
 Mello, M. L. S. 653  
 Mellouk, Z. 77  
 Meloni, A. 832  
 Meloni, M. 123  
 Mendes, G. L. C. 604  
 Menditto, E. 945  
 Menegazzi, M. 470  
 Menegazzo, L. 126  
 Meneghini, L. F. 926, 957  
 Menghini, R. 64, 159, 160, 643, 1191  
 Menoud, V. 214, 216  
 Mensink, M. 598  
 Menzaghi, C. 136  
 Mercader, J. M. 295, 658  
 Mercou, M. E. 605  
 Merker, L. F. 374  
 Merletti, F. 324  
 Merlotti, D. 1085  
 Mersebach, H. 615  
 Mesa, J. 1267  
 Mesangeau, D. 1215  
 Meses, L. 1118  
 Messinger, D. 1036  
 Metra, M. 124  
 Meugnier, E. 209  
 Mewes, H.-W. 635  
 Meyer, A. 501, 512  
 Meyers, J. L. 797, 936, 981  
 Meyers, L. 457  
 Mezzetti, A. 945  
 Mi, S. 229  
 Milek, T. 703  
 Mianowska, B. 1237  
 Miao, S. 31  
 Miao, Z. 31  
 Miao Jonasson, J. 51  
 Miccoli, R. 291, 346, 1192, 1196  
 Michael, M. D. 24, 819  
 Michael, M. D. 547, 914  
 Michalski, M.-C. 667  
 Michau, A. 414  
 Michels, A. 457  
 Micó, J. A. 48  
 Midthjell, K. 85, 168, 317, 320  
 Miele, C. 545  
 Miglio, G. 1123  
 Mikkola, K. 1261  
 Milà, M. 164  
 Milad, M. 1110  
 Milata, V. 879  
 Milburn, M. 108  
 Milczarczyk, A. 871  
 Milek, T. 236  
 Milicic, T. 180, 475  
 Miliou, A. 1129  
 Miller, K. 969  
 Miller, R. G. 185  
 Miller, S. 804  
 Milochau, A. 397  
 Milovanovic, I. 321  
 Mimenza, A. J. 1179  
 Mimoso, J. 58, 59  
 Min, K. W. 851, 852, 1177  
 Miñambres, I. 612  
 Minami, K. 421  
 Minardi, V. 309  
 Mindlova, M. 1029  
 Minehira, K. 20  
 Mineoka, Y. 568  
 Mingrone, G. 584, 658  
 Minimo, L. 111  
 Minuchin, G. 603  
 Miossec, P. 815  
 Miranda, M. L. 604  
 Miranda-Palma, B. 910, 911  
 Mirasierra, M. 508  
 Mirra, P. 545  
 Mirza, A. 110  
 Misnikova, I. V. 792  
 Misra, S. 984  
 Mistodie, C. 940  
 Mitchell, Y. 1235  
 Mitchell, B. 49  
 Mitchell, P. N. 1140  
 Mithal, A. 628  
 Mithal, A. 732  
 Mitterer, G. 671  
 Miura, J. 165  
 Miyajima, A. 63  
 Miyamoto, L. 894  
 Miyamoto, S. 62, 1115  
 Miyaoka, T. 525  
 Miyashita, Y. 171  
 Miyata, T. 1255  
 Miyata, Y. 282, 469  
 Miyazawa, K. 1108  
 Miyoshi, H. 140, 161  
 Mo, Y. 977  
 Mobasher, M. A. 94  
 Möckel, S. 260  
 Modder, J. 1089  
 Modi, K. D. 732  
 Mohammad, I. 621  
 Mohammedi, K. 153  
 Mohan, V. 793  
 Mohan, V. 851  
 Mojibian, M. 443  
 Molas, M. 418, 448  
 Molinari, C. 198  
 Möller, G. 670  
 Möller, J. B. 460  
 Møller, N. 95  
 Mollet, I. 97, 486  
 Molnár, G. 1144  
 Molven, A. 625  
 Molz, E. 898  
 Monami, M. 343, 1171  
 Mondon, P. 262  
 Monill, J. 612  
 Monnet, A. 1215  
 Monnier, V. M. 1174  
 Montalto, G. 782  
 Montanya, E. 427  
 Monteiro, E. C. 93  
 Monteiro, M. 1096  
 Monteleone, G. 64  
 Montero, C. 819  
 Monterrat, C. 397  
 Montgomery, M. K. 638, 649  
 Montori, V. 1019  
 Montrose-Rafizadeh, C. 547  
 Moon, H. 1177  
 Mooney, E. 1172  
 Moore, M. C. 24  
 Moore, W. V. 454  
 Moran, A. 454  
 Morano, S. 1139  
 Morcillo, S. 672, 870  
 Moreno, E. 1061  
 Moreno, M. 562  
 Moreno-Asso, A. 144  
 Moreno-Navarrete, J. M. 208, 295, 562, 658, 676  
 Moreno-Villegas, Z. 634  
 Moretti, S. 495  
 Morgado, C. 1182  
 Morgan, N. G. 89, 492  
 Morganti, R. 1180  
 Mori, I. 589, 927  
 Mori, K. 733  
 Morikawa, Y. 63, 686  
 Morimoto, A. 348, 844, 884, 950  
 Morino, G. S. 901  
 Morio, B. 209  
 Morita, H. 589, 927  
 Morita-Ohkubo, T. 171  
 Moriya, T. 1101  
 Morkrid, K. 882, 1071  
 Morley, A. 151  
 Morris, A. 990  
 Morris, D. H. 311  
 Morro, M. 418, 444, 448  
 Morrow, L. A. 742, 747, 908, 916, 925  
 Mortellaro, M. 1030  
 Mortensen, B. 561, 592  
 Mortensen, H. B. 238, 275, 276, 614, 963, 1015  
 Mosdøl, A. 882  
 Mosenzon, O. 119  
 Moser, S. 1269  
 Mospan, B. 871  
 Moss, C. E. 576  
 Mostafa, S. A. 311  
 Motas, S. 191  
 Motoshima, H. 661  
 Motta, D. F. 604  
 Mounier, C. 1266  
 Mounier, C. 553  
 Moura, L. 1169  
 Mouraux, T. 267  
 Moussa Ali, H. 1266  
 Movassat, J. 428, 714  
 Moysakis, I. 1241  
 Moyssset, P. 730  
 Mozas, D. 166  
 Mpotsios, K. 56  
 Msihid, J. 813, 814  
 Mstsuda, H. 718  
 Mu, P. 368  
 Muchmore, D. B. 41, 907, 908  
 Mudaliar, S. 244  
 Muehlenbartmer, I. 810  
 Mueller, G. 1156  
 Muenzner, M. 210  
 Mughal, S. 1270  
 Muir, K. R. 429  
 Mukhopadhyay, S. 873  
 Mulder, H. 97, 98, 176  
 Müller, A. 1036  
 Müller, A. 410  
 Müller, M. J. 974  
 Müller, N. 952, 959, 1098, 1205  
 Müller, R. 1269  
 Müller, U. A. 952, 959, 998, 1098, 1205  
 Müller-Wieland, D. 22  
 Mulligan, B. 398  
 Mullooly, N. 521  
 Muñoz, J. 340  
 Muñoz, S. 633  
 Muñoz-Mediavilla, C. 1258  
 Munsaka, M. 839  
 Münster, C. 398, 410  
 Murai, M. 847  
 Murakami, T. 219  
 Muraoka, A. 363  
 Murillo, S. 933  
 Murphy, J. 1090  
 Murphy, M. 1172  
 Murphy, N. J. 1211, 1252  
 Musi, N. 563  
 Mustafa, E. 79, 1064  
 Muto, A. 143  
 Muto, T. 190  
 Muzik, J. 1128  
 Mziaut, H. 398  
 N  
 Nabi, I. R. 67  
 Nabrdalik, K. 657  
 Nada, E. 57  
 Nadeev, A. P. 1134  
 Nadir, D. 370, 377  
 Nagao, M. 503, 1257  
 Nagar, R. 118  
 Nagashima, H. 505  
 Nagashima, M. 778  
 Nagashima, Y. 705  
 Nagashimada, M. 636  
 Nagorny, C. L. 99  
 Nagorny Holmberg, C. L. F. 176  
 Nahar, Q. 892  
 Naito, E. 1247



- Nakagami, T. 362  
 Nakagawa, T. 894  
 Nakajima, Y. 503, 1257  
 Nakamura, A. 705, 864  
 Nakamura, J. 1107, 1108, 1247  
 Nakamura, K. 1044, 1046, 1051  
 Nakamura, N. 1107, 1108  
 Nakamura, N. 258, 568  
 Nakamura, S. 282  
 Nakamura, S. 949  
 Nakamura, T. 816  
 Nakamura, Y. 172  
 Nakamura, Y. 179, 648  
 Nakata, M. 488  
 Nakata, S. 282, 469  
 Nakatani, M. 1147  
 Nakaya, Y. 446  
 Nakayama, H. 257  
 Nakstad, B. 1071  
 Nam-Goon, I. S. 852  
 Nan, R. 877  
 Nandy, D. 1056  
 Nanjo, K. 1147  
 Nannipieri, M. 108, 583  
 Napoli, N. 115  
 Narayan, K. 420  
 Nardi, S. 830  
 Naruse, K. 1107, 1108  
 Naselli, G. 424  
 Naskret, D. 163, 227  
 Nasser, A.-D. 886  
 Nasteska, D. 179  
 Nastesuka, D. 363  
 Nata, K. 426  
 Natali, A. 108, 610  
 Natalicchio, A. 125, 513, 675, 1260  
 Natasha, K. 975  
 Nathan, D. M. 1105  
 Nauck, M. A. 2, 246, 721  
 Naujok, O. 215  
 Navales, G. 1171  
 Navarro, M. 419  
 Naver, H. 37, 915  
 Naver, L. 1245  
 Navis, G. J. 135, 345  
 Neal, B. 764  
 Neal, D. 37  
 Neelakantham, S. 843  
 Neerup, T. S. R. 177, 823  
 Negro, F. 389, 639  
 Nelson, C. 995  
 Nemani, M. 553  
 Nemoto, H. 894  
 Nerup, J. 281  
 Nesca, V. 216  
 Neschen, S. 635  
 Netea, M. G. 66, 560, 659  
 Neto, M. G. 9  
 Neubacher, D. 848, 849, 1233  
 Neufeld, Z. 412  
 Neukamm, S. S. 542  
 Neumann, U. H. 570  
 Neuper, C. 453  
 Neutsky-Wulff, A. V. 114  
 Nevado, C. 387  
 Nevalainen, J. 265, 326  
 Neville, M. 629  
 Newhouse, J. 1019  
 Newman, J. 795  
 Newsholme, P. 412, 521  
 Ng, B. 361  
 Ng, J. 1187  
 Ng-Mak, D. S. 719, 720  
 Nguema, J.-L. 1013  
 Nguyễn, M.-T. 32, 1199, 1206  
 Ni, L. 1125  
 Ni, Y. 636  
 Nica, A. C. 139  
 Niccoli, G. 1221  
 Nichols, G. A. 181, 353  
 Nicod, N. 286  
 Nicolucci, A. 249, 968, 1020, 1139  
 Niedzwiecki, P. 227  
 Nielsen, A. A. 253, 1164  
 Nielsen, J. S. 600  
 Nielsen, K. E. 253, 1016, 1164  
 Nielsen, L. B. 370, 377  
 Nielsen, L. B. 238, 275, 276, 614  
 Nielsen, R. R. 95  
 Nielsen, S. E. 1132, 1146, 1244  
 Niemann, J. 595, 596  
 Niemoeller, E. 3, 810, 815  
 Niessen, H. W. M. 61  
 Niessen, M. 388  
 Niessen, P. M. G. 61, 1255  
 Niessner, F. 481  
 Nieuwdorp, M. 644, 65  
 Nigro, C. 545  
 Nigro, P. 675  
 Nihat, A. 1001  
 Niinistö, S. 265, 326  
 Niiya, T. 973  
 Nijpels, G. 133, 234, 285, 354, 988, 1002  
 Niki, I. 401  
 Nikita, K. 1080  
 Nikitina, L. D. 466  
 Nikolic, N. 29  
 Nikolic, I. 497, 647  
 Nikonova, T. V. 465  
 Nils, E. 51  
 Nilsson, L.-G. 14  
 Nilsson, P. M. 690  
 Ning, G. 1009  
 Ning, M. 632, 817  
 NIRAD Study Group 269  
 Nishi, M. 1147  
 Nishibata, I. 1073  
 Nishikawa, T. 661  
 Nishimura, E. 37, 915  
 Nishimura, R. 348, 844, 884, 950  
 Nisio, S. 831  
 Niskanen, L. 763  
 Niswender, K. 939  
 Nitert, M. D. 105  
 Nizard, J. 1091  
 Nizzoli, M. 1165  
 Njølstad, P. R. 625  
 Noctor, E. 79, 1077  
 Noda, M. 1197  
 Nodale, M. 194, 195  
 Noel, C. 434  
 Nogi, Y. 778, 786  
 Noh, J. 1143  
 Nolan, J. J. 364, 602  
 Nolden, T. 27  
 Nomoto, H. 140  
 Nomura, H. 1197  
 Nora, M. 1096  
 Nordestgaard, B. G. 344  
 Nordwall, M. 1099  
 Nørgaard, K. 1058  
 Norkova, J. 476  
 Norris, J. M. 106, 268  
 Nosaka, T. 686  
 Nosek, L. 909, 912  
 Nosiglia, G. 1072  
 Noso, S. 650  
 Nosso, G. 586  
 Notomi, K. 778, 786  
 Novara, F. 336  
 Novelli, M. 470  
 Novellino, E. 945  
 Novials, A. 121, 144, 436, 500, 539, 540, 933  
 Nóvoa, F. J. 328  
 Nóvoa, J. 1258  
 Nowaczyk, M. 236  
 Nowak, N. 333, 1065  
 Nowak, W. N. 254  
 Nowotny, B. 310  
 Nowotny, P. 22, 129, 131, 445, 559  
 Nulle, F. 128, 595, 596  
 Nummenmaa, L. 28  
 Nuti, R. 1085  
 Nuutila, P. 8, 28, 565, 601, 1261  
 Nybäck-Nakell, Å. 796  
 Nyberg, I. 119  
 Nyberg, L. 14  
 Nybo, L. 217  
 Nygaard, H. 1160  
 Nyström, T. 1189
- O**
- O Hara, M. 999  
 O'Brien, T. 1172, 1263  
 O'Connor, P. J. 353, 979  
 O'Farrell, L. S. 25  
 O'Gorman, D. J. 602  
 O'Grady, T. 364  
 O'Hanlon, D. J. 602  
 O'Hare, J. P. 1017  
 O'Loughlin, A. 1172  
 O'Neal, D. N. 1054  
 O'Neill, C. 1095  
 O'Shea, P. 1077  
 Obach, M. 444, 447, 448  
 Obara, A. 648, 708  
 Obendorf, F. 134  
 Oberholzer, J. 438  
 Obrosova, A. A. 1174  
 Obrosova, I. G. 1121, 1174  
 Occhipinti, M. 150  
 Ochiai, H. 742  
 Ochoa, B. 397  
 Ocon-Bretón, J. 1103  
 Odawara, M. 858  
 Ofstad, A. P. 1226  
 Ogasawara, H. 739  
 Ogata, M. 335, 337  
 Ogawa, A. 973  
 Ogawa, D. 33, 62, 1115  
 Ogawa, K. 1147  
 Ogawa, W. 816  
 Ogawa, Y. 718  
 Ogbaa, I. 772, 773, 775  
 Ogilvie, P. 7  
 Ogunnowo-Bada, E. 576, 622  
 Ogura, M. 648  
 Oh, K. 1131, 372  
 Oh, T. 1127  
 Ohashi, A. 648  
 Ohashi, K. 426  
 Ohashi, Y. 319, 1243  
 Ohlsson, C. 1213  
 Ohlsson, L. 828  
 Ohru, T. 1197  
 Ohsawa, N. 783  
 Ohsugi, M. 460, 734  
 Oikawa, S. 503, 1257  
 Oikonen, V. 1261  
 Oka, Y. 143  
 Okabe, E. 1124, 1212  
 Okada, H. 589  
 Okada, H. 568  
 Okada-Iwabuchi, M. 113  
 Okazaki, Y. 430  
 Okazaki, Y. 734  
 Oke, J. 789  
 Okerson, T. 719, 720  
 Okita, K. 282, 423  
 Okizaki, S. 1101  
 Okuda, M. 1101  
 Olšovský, J. 152  
 Olaizola, I. 164  
 Ølgaard, J. 1223  
 Olivares, R. A. 1179  
 Oliveira, C. L. 987  
 Oliveira, J. 500  
 Oliyarnik, O. 551, 1152  
 Olsson, A. 105  
 Olsson, L. 168  
 Olsson, T. 14  
 Omar, B. 861  
 Omi, M. 1108  
 Onda, Y. 348  
 Ongen, H. 139  
 Onishi, S. 1124, 1212  
 Onishi, Y. 949  
 Ono, M. 718  
 Ono, M. 1100  
 Ono, Y. 949  
 Oppert, J.-M. 695  
 Oram, R. A. 463  
 Orchard, T. J. 185  
 Orečná, M. 438  
 Orešič, M. 87  
 Orho-Melander, M. 288, 290, 869  
 Oriente, F. 637  
 Orime, K. 705  
 Orlando, M. R. 125, 513, 1260  
 Orloff, D. 822  
 Orlov, U. U. 1185  
 Orsi, E. 1139  
 Ortega, F. J. 208, 295, 562, 658, 676  
 Örtqvist, E. 238, 278  
 Ortsäter, H. 519, 535  
 Osawa, M. 165  
 Osborne, B. 638  
 Osborne, E. 547  
 Osei, K. 527  
 Ost, M. 26, 130  
 Östenson, C.-G. 893  
 Oster, P. 997  
 Østergaard, J. A. 1130  
 Osterhoff, M. A. 548, 550, 712  
 Österholm, A.-M. 170  
 Östgren, C. 690  
 Östman, E. 574  
 Østoft, S. H. 334  
 Ota, H. 525  
 Ota, T. 636  
 Otabe, S. 257  
 Otero, Y. F. 387  
 Ott, J. 542  
 Oudemans-van Straaten, H. M. 184  
 Quertani, H. 875  
 Out, C. 644  
 Ouwers, D. M. 92, 129, 543, 654, 1219  
 Overgaard, A. J. 281

- Overgaard, R. V. 460  
 Owada, Y. 512  
 Owczarek, A. 657  
 Owen, K. R. 332  
 Owens, D. R. 983, 1142  
 Owens, L. A. 1069, 1087  
 Owens, P. 1263  
 Owens, R. A. 120  
 Own, N. 905  
 Oyen, W. J. G. 263  
 Ozawa, S. 1108
- P**
- Pääkkönen, M. 722  
 Pabinger, I. 727  
 Pacal, L. 1128  
 Pácal, L. 152  
 Pach, P. F. 569  
 Paciello, O. 637  
 Pacini, G. 310, 828  
 Padmaja, R. 547  
 Padwal, R. S. 696  
 Paeth, G. 507, 1081  
 Paez, A. M. 711  
 Pagacova, L. 255  
 Pagano, T. B. 637  
 Pagliarino, A. 232, 701  
 Pais, R. 717  
 Palalau, A. I. 1090  
 Palerm, C. C. 1050  
 Palermo, A. 115  
 Pallardo Sánchez, L. F. 84  
 Pallone, F. 64  
 Palmer, C. N. A. 50  
 Palmer, J. 956  
 Palmieri, F. 523  
 Palming, J. 549  
 Palmisano, G. 404  
 Palou, A. 208  
 Pamplona, R. 130  
 Pan, C. 948  
 Pan, J. 1209  
 Pandit, A. 1172  
 Pandolfi, A. 1249  
 Panduru, N. M. 154, 1135  
 Panoutsopoulos, G. 56  
 Panunzi, S. 351  
 Paoli, A. 730  
 Papadimitriou, A. 1080  
 Papadogiannis, D. 1129, 1241  
 Papadopoulos, G. K. 474, 477  
 Papageorgiou, N. 1129  
 Papamargaritis, D. 9  
 Papanas, N. 44, 1157  
 Papantoniou, S. 1157  
 Papaioikonomou, S. 1129  
 Papassotiropoulos, I. 1082  
 Papatheodorou, D. 1082  
 Papós, M. 1158  
 Papp, A. 60  
 Pappas, A. 86  
 Parasuraman, S. 181  
 Pardo, G. 295, 658, 676  
 Pardo, R. 127  
 Pardo, S. 993  
 Pardo, V. 94  
 Paredes, E. M. 439  
 Parfentjeva, E. M. 1134  
 Paries, J. 1215  
 Parikh, S. J. 242, 721, 743, 744, 745, 748, 751, 753, 754, 755, 756, 758, 777  
 Paris, I. 1122
- Park, C. 372, 1131  
 Park, I. 1177  
 Park, J. 1143  
 Park, J. 509, 889, 1151  
 Park, J.-Y. 355, 851  
 Park, K.-S. 1177  
 Park, K.-Y. 1250  
 Park, M. 509, 889, 1151  
 Park, S. 372, 1131  
 Park, S. 1131, 1177  
 Park, S. 1177  
 Park, S.-W. 1242  
 Park, S. 372  
 Parker, H. E. 576  
 Parkes, J. L. 993  
 Parkin, C. G. 953, 982, 1005, 1035, 1040  
 Parkkola, R. 28, 601  
 Parkkonen, M. 170  
 Paroni, F. 467, 522  
 Parra Ramírez, P. 84  
 Parrillo, L. 637  
 Parthasarathy, N. 793  
 Partke, H.-J. 22, 129, 445  
 Parveen, T. 892  
 Parving, H.-H. 1146, 1223  
 Paschou, S. A. 474, 477  
 Passaretti, F. 651  
 Passera, P. 1163  
 PASSI Coordinating Group 309  
 Passlack, W. 654  
 Paster, I. P. 458  
 Pataky, Z. 1190  
 Patel, A. G. 728  
 Patel, D. 1089  
 Patel, J. 1001  
 Patel, N. 995  
 Patel, R. N. 359  
 Patel, S. 6, 148, 847, 848, 849, 850, 1233  
 Patel, S. A. 591  
 Patel, V. 752  
 Patois-Vergès, B. 1224  
 Patrakeeva, E. 1049  
 Patsouris, D. 667  
 Patti, A. 104, 431, 468  
 Patti, A. M. 782  
 Patti, L. 76, 878  
 Pattou, F. 391, 434, 520  
 Paufler, A. 413  
 Paul, D. L. 406  
 Paul, S. K. 618, 621  
 Paulo, C. P. 987  
 Pávics, L. 1158  
 Pavkovic, P. 1150  
 Pavlik, T. 1128  
 Pawlowski, M. 928, 931, 934  
 Paya, A. 1076  
 Pazderska, A. 602  
 Pe Benito, M. 749  
 Pearson, E. R. 50  
 Pearson, G. 502  
 Pecenkova, V. 1152  
 Pedersen, B. K. 407, 460, 546, 561, 600  
 Pedersen, I. D. 262, 442, 481  
 Pedersen, J. 409, 713  
 Pedersen, M. 460  
 Pedersen, O. 297, 334, 1223  
 Pedersen, S. B. 296, 666  
 Pedersen-Bjergaard, U. 138, 221, 279, 620  
 Pedro, J. R. 557  
 Peerrally, Z. 292
- Pekareva, E. V. 465  
 Peled, D. 455  
 Pelikanova, T. 74, 75  
 Pellegrini, F. 136, 249  
 Pellegrini, M. A. 1020  
 Pelletier, C. 350  
 Peña, V. 833  
 Peña Agüera, A. 293  
 Pencek, R. 4, 804  
 Penfold, S. A. 151  
 Penforis, A. 845  
 Pengilly, C. 995  
 Penno, G. 1139, 1196  
 Pensini, C. 901  
 Perakakis, N. 507, 1081  
 Perego, C. 478, 495  
 Pereira, I. M. 987  
 Pereira, M. J. 549  
 Pereira, S. 59  
 Perez, A. 704, 787, 1228, 1229, 1230, 1231  
 Pérez, A. 612  
 Pérez, C. 612  
 Perez, F. 1225  
 Perez Nevot, B. 293  
 Perez-Cadena, Z. 791  
 Perimenis, P. 81  
 Perkovic, V. 36, 764  
 Péronet, C. 23  
 Perrea, D. 366, 724, 867, 1109  
 Perrelli, A. 1020  
 Perren, A. 737  
 Perrin, E. 1033  
 Perrini, S. 125, 513, 675, 1260  
 Perron, P. 82  
 Perrone, G. 830  
 Perrot, S. 48  
 Persaud, S. J. 378, 379, 380, 381, 385, 396  
 Persson, F. 1146, 1223, 1244  
 Persson, S. 1052  
 Perticone, F. 198  
 Pesau, G. 134  
 Peschechera, A. 125, 675  
 Peschel, S. 44  
 Peschiaroli, A. 643  
 Petegnief, V. 539  
 Peter, A. 685, 1081  
 Peter, R. 983  
 Péterfai, É. 569  
 Peterkova, V. 623  
 Peters, A. 308  
 Peters, A. 800  
 Peters, K. E. 1200  
 Petersen, B. 953, 1005, 1035  
 Petersen, J. S. 238, 276  
 Petersen, S. T. 114  
 Petersen, W. 262  
 Peterson, C. A. 695  
 Peterson, K. 879  
 Petersson, S. J. 594  
 Petkova, K. V. 1084  
 Petrak, F. 974  
 Pétremand, J. 516  
 Petri, K. C. 805  
 Petrie, J. L. 608  
 Petrie, J. R. 2  
 Petrou, S. 1017  
 Petrov, P. 208  
 Petry, T. 9  
 Petsiou, A. N. 474, 477  
 Pettus, J. 993  
 Petzold, A. 331  
 Peyrot, M. 222, 955, 967
- Peyrou, M. 639  
 Peyser, T. 1051  
 Pezullo, J. 47  
 Pfarr, E. 770  
 Pfeiffer, A. F. H. 260, 548, 550, 694, 707, 712  
 Pfeiffer, C. 808  
 Pfister, M. 747, 749  
 Pflueger, M. 895  
 Pflüger, M. 87, 325  
 Pfutzner, A. 118  
 Pham, I. 1206  
 Pham, T. T. 8, 565  
 Pharisien, I. 1094  
 Phieler, J. 158  
 Phielix, E. 22, 129, 131, 559  
 Philippe, E. 887  
 Philippe, J. 259, 1190  
 Philippou, G. 1063  
 Philis-Tsimikas, A. 39, 40, 920  
 Philotheou, A. 946  
 Phippard, D. 147  
 Piaggese, A. 1186  
 Piatkiewicz, P. J. 236, 703  
 Pibernik-Okanovic, M. 251  
 Picardi, P. K. 555  
 Piccinini, M. N. 729, 735  
 Picconi, A. 124  
 Pieber, T. R. 453, 924  
 Piekarski, R. 899, 904  
 Pierre, B. 1224  
 Pierreisnard, A. 710  
 Pietraszek, A. 296, 666  
 Pietrzak, I. 1237  
 Pihlajamäki, J. 722  
 Pijl, H. 611  
 Pilacinski, S. 163  
 Pilavachi, E. J. 1252  
 Pildava, S. 699  
 Piletić, M. 939  
 Pilgaard, K. 592  
 Pillegand, C. 1206  
 Pillot, B. 553  
 Pilorget, V. 902, 947  
 Pina, E. 58, 59  
 Pinach, S. 655, 1133  
 Pinckney, A. 454  
 Ping, L. 3, 807  
 Pinget, M. 23, 810  
 Pini, A. 1123  
 Pinnetti, S. 6  
 Piot, C. 55  
 Pipeleers, D. G. 267  
 Pippa, A. 470  
 Pirags, V. 1236  
 Piro, S. 577  
 Pirot, N. 72  
 Piske, M. 534  
 Pitale, S. 851  
 Pitocco, D. 1221  
 Piva, F. 281  
 Pivovarova, O. 260, 707  
 Piya, M. K. 1270  
 Pizarro, E. 164  
 Pizarro-Delgado, J. 406  
 Place, J. 196, 1029  
 Plamboeck, A. 248, 582, 713  
 Platten, I. 1039  
 Platzer, M. 590  
 Pleus, S. 1043  
 Pliszka, J. 689, 1086  
 Plomgaard, P. 407, 546  
 Plotka, M. 173  
 Plotkin, D. J. 822

- Pochinka, I. 230  
 Pociot, F. 275, 276, 281  
 Poduri, M. 794  
 Poerksen, S. 275  
 Poggi, M. 157  
 Pohl, R. 906  
 Pokka, T. 240  
 Polak, B. C. 234  
 Polidori, D. 244, 761  
 Polkinghorne, K. 1105  
 Polyzou, E. 1081  
 Pons, B. 725  
 Ponsati, B. 1112  
 Ponticello, R. 752  
 Poon, W. 284  
 Poongothai, S. 793  
 Poopalasundaram, S. 392  
 Pop, V. J. M. 252  
 Porcellati, F. 938  
 Porchay-Baldérelli, I. 289  
 Porcher, R. 80  
 Pörksen, S. 238, 276  
 Porozov, S. 470  
 Porras, N. 870  
 Porta, M. 1163  
 Porter, D. 579  
 Porter, L. 4, 825  
 Portero-Otín, M. 130  
 Portha, B. 428, 714, 887  
 Portron, A. 767  
 Porzio, O. 1191  
 Possenti, V. 309  
 Post, M. 1255  
 Potasso, L. 1081  
 Poulsen, P. 460, 558, 592  
 Poulton, J. 173  
 Pournaras, D. J. 9  
 Pournourmohammadi, S. 408  
 Pouwer, F. 252  
 Powell, D. 772, 774, 775, 776  
 Powers, C. 136  
 Powers, J. P. 31  
 Poy, M. 213  
 Poy, P. 701  
 Pozzilli, P. 115, 145, 455  
 Pradas-Juni, M. 286  
 Pralle, K. 1036  
 Prasad, T. 674  
 Prasek, M. 1150  
 Pratley, R. 841  
 Pratt, E. 818  
 Pravenec, M. 551  
 Prázný, M. 200  
 Pregnancy and Diabetes Study Group from the Portuguese Society of Diabetology 1088  
 Prellberg, M. 680  
 Prentki, M. 216  
 Prevost, A. T. 183  
 Price, D. 1046, 1051  
 Priebe, S. 590  
 Prietl, B. 453  
 Prieur, X. 553  
 Primo, M. E. 410  
 Prince, M. J. 916, 917, 918, 919  
 Prince Mutaib Chair for Biomarkers of Osteoporosis 886  
 Prinster, A. 76  
 Prinzis, T. 137  
 Prischl, F. C. 1138  
 Prnjavorac, B. 630  
 Procner- Czaplińska, M. 970, 1053  
 PROGENS Study Investigators 871  
 Prokisch, H. 103, 294  
 Prokofev, S. A. 1185  
 Protogerou, A. 1241  
 Prusty, V. 951  
 Ptaszynska, A. A. 242, 721, 743, 744  
 Pucci, L. 1196  
 Puddu, A. 575  
 Puder, J. 1011  
 Pueyo, N. 295, 658, 676  
 Pugliese, G. 1139  
 Puglisi, F. 675  
 Puig, J. 1076  
 Puig-Domingo, M. 164  
 Pujals, M. 1193  
 Puk, O. 505  
 Pulizzi, N. 1192  
 Pulkkinen, L. 722  
 Pullen, T. J. 101, 212  
 Purrello, F. 577  
 Pusalkar, P. 1001  
 Pussinen, P. 327  
 Putignano, D. 945  
 Puyal, J. 516  
 Pye, S. 606  
**Q**  
 Qian, H.-R. 120  
 Qiu, S. 1145, 994  
 Qiu, W. 52  
 Qiu, Y. 1119  
 Qiu, Y. 368  
 Qiu, Y. 985  
 Qu, Y. 916, 917, 918, 919  
 Quadri, R. 1163  
 Querci, F. 729, 735  
 Quéré, S. 845  
 Quintanilla-Martinez, L. 459  
 Quintin, D. 434  
 Quirós, C. M. 479  
 Quistorff, B. 592  
 Quotb, A. 484  
**R**  
 Rabelink, T. J. 433  
 Rabøl, R. 949  
 Rabuazzo, A. M. 577  
 Raccach, D. 815  
 Rackham, C. L. 432, 440  
 Raddatz, K. 21  
 Radisic Biljak, V. 1150  
 Radulian, G. 877  
 Radziuk, J. 606  
 Raffaitin, C. 1246  
 Ragonesi, P. D. 735, 829  
 Rahman, A. 1232  
 Raila, J. 210  
 Raimondo, A. 174  
 Rainone, A. 651  
 Raja, P. 1037, 1038  
 Rajan, W. 416  
 Rajaraman, S. 1030  
 Rajkovic, N. 180, 475  
 Rajput, R. 367  
 Raluca, N. 1198  
 Rami, B. 1066  
 Ramirez, L. 191  
 Ramona Maria, D. 1198  
 Ramos, S. 528  
 Ramos-Álvarez, I. 634  
 Ramotowska, A. 1053  
 Ramracheya, R. 70, 487  
 Ramshaw, H. S. 534  
 Ramstetter, E. 1043  
 Ran, J. 1116  
 Rana, A. 222, 955  
 Ranasinghe, P. 1097  
 Ranasinha, S. 1105  
 Ranc, K. 700  
 Rangel, D. 1225  
 Rani S. N. S. 547  
 Rao, G. 1154  
 Raoux, M. 484  
 Raposo, J. F. 292, 377, 987  
 Raschert, J. 544  
 Rasenack, R. 1081  
 Rasmussen, A. 253, 1164  
 Rasmussen, K. 592  
 Rasmussen, M. A. 276  
 Rasmussen, S. 40, 617  
 Rasmussen, T. 88  
 Rasouli, B. 270, 317, 320  
 Rastaldi, M. 1133  
 Rathe, J. Ø. 271  
 Rathjen, T. 213  
 Rathmann, W. 103, 294, 307, 308, 316, 347, 784, 854, 1161  
 Rathsmann, B. 1189  
 Ratner, R. E. 38, 581, 615, 806, 957  
 Ravaut, C. 415  
 Ravier, M. A. 72, 101  
 Raviolo, A. 1163  
 Ravussin, E. 80  
 Rayanagoudar, G. 1140  
 Raymond, N. 1017  
 Rayner, C. K. 247, 820  
 Rayner, N. W. 694  
 Raz, I. 118, 119, 145, 303, 304, 455  
 Rebas, P. 725  
 Rebuffat, S. 500, 684  
 Recasens, A. 991  
 Reed, L. J. 728  
 Reers, C. 385  
 Rees, M. G. 174  
 Reeves, H. L. 291  
 Reeves, N. D. 1178  
 Regazzi, R. 211, 214, 216  
 Regife, V. 1141  
 Rehfeld, J. F. 574  
 Rehorova, J. 152, 1128  
 Reichard, M. 43  
 Reichetzeder, C. 35  
 Reid, R. J. 353  
 Reifel Miller, A. 547  
 Reimann, F. 576, 716  
 Rein, P. 339  
 Reinbeck, A. L. 129, 445  
 Reinbothe, T. M. 482  
 Reinhard, H. 1146, 1244  
 Reis, F. 557  
 Remer, T. 316, 881  
 Ren, Z. 368  
 Renal Insufficiency And Cardiovascular Events (RIACE) Study Group 1139  
 Renard, E. M. 194, 196, 1029  
 Renard, M. 1076  
 Renaud, S. 484  
 Renaut, L. 262  
 Renner, S. 505  
 Rensen, S. S. 61  
 Renström, E. 482  
 Repice, M. 419  
 Repina, E. A. 472, 1185  
 Resi, V. 1072, 1093  
 RESTORE Extension Group 192  
 RETAIN Study Team 147  
 Reurean-Pintilei, D. 607  
 Revuelta-Cervantes, J. 1118  
 Rewers, A. 323  
 Rewers, M. 106, 268, 323  
 Rex, J. 854  
 Rezanian, A. 420  
 Rezaninová, L. 256  
 Rezende, L. F. 386  
 Reznick, J. 638  
 Reznik, Y. 989  
 Rhee, B. 1143  
 Rhee, B. 509, 1151  
 Rhee, B. 889  
 Rhee, E. J. 372, 852, 1131  
 Riant, E. 566  
 Riasniy, V. 450  
 Ribeiro, M. J. 93, 554  
 Ribeiro, R. 610  
 Ribel-Madsen, R. 297  
 Ribera, A. 191  
 Ribot, J. 208  
 Ricart, W. 208, 295, 562, 658, 676  
 Riccardi, G. 76, 586, 878, 945  
 Richards, C. J. 1142  
 Richardson, S. J. 89  
 Riches, C. H. 576, 622  
 Richter, D. 410  
 Richter, E. A. 217, 588  
 Ricotti, A. 711  
 Riddle, M. 3, 807  
 Ridolfi, V. 124  
 Rieckmann, A. 98  
 Riegler, S. 945  
 Rieusset, J. 532, 667  
 Rigalleau, V. 710, 876, 1246  
 Rigby, M. R. 147, 454  
 Rijzewijk, L. J. 1219  
 Rimmer, N. 507  
 Ringholm, L. 83  
 Riopel, M. 422  
 Riphagen, I. J. 135, 155  
 RISC Investigators 306  
 RISC Study Group 108  
 Risérus, U. 78  
 Ristow, M. 590  
 Rita, R. 405  
 Rittig, K. 683  
 Riva, M. 207  
 Riveline, J.-P. 80  
 Rivellese, A. A. 76, 878  
 Rivera, J. F. 538  
 Rivieccio, A. 878  
 Rizell, M. 549  
 Rizza, R. A. 585  
 Rizzo, L. 1186  
 Rizzo, M. 782  
 Rizzuto, R. 101  
 Robert-Vila, M. 991  
 Robertson, K. J. 963  
 Robin, I. 1224  
 Roca, C. 447  
 Rocha, G. 1096  
 Roche, B. 1003  
 Rockova, V. 656  
 Rockstroh, D. 678  
 Rodbard, H. W. 39, 617, 920  
 Roden, M. 22, 44, 103, 129, 131, 294, 310, 316, 445, 559  
 Rodrigues-Carvalho, A. 1117  
 Rodriguez, L. 12  
 Rodriguez, M. 603, 947



- Rodriguez, N. 419  
 Rodríguez Vitoria, J. 603  
 Rodriguez-Hermosa, J. I. 295  
 Roep, B. O. 451  
 Roepstorff, C. 909, 925  
 Rogazzo, M. 660  
 Rogers, K. 424  
 Roglic, G. 1204  
 Rogner, U. C. 283  
 Rohwedder, K. 750, 753, 755  
 Roivainen, M. 331  
 Rojo-Martinez, G. 672, 870  
 Rokadiya, S. 1001  
 Rolandsson, O. 14  
 Roliński, J. 899, 904  
 Rölver, K.-M. 962  
 Romagnoli, F. 1171  
 Roman, E. R. 556  
 Romano, D. 829  
 Romanov, V. V. 1134  
 Romero, M. 406  
 Romero-Afrima, L. 303, 304  
 Romijn, J. A. 1219  
 Romon, I. 321  
 Roncero, I. 552  
 Rondas, D. 402  
 Rondinelli, M. 336  
 Rondinini, L. 150  
 Rönn, T. S. 105, 107  
 Ronningen, K. S. 88  
 Roop, J. 37  
 Roper, D. 984  
 Rorsman, N. 70  
 Rorsman, P. 70, 486, 487  
 Ros, M. 94  
 Ros, S. 23  
 Rosa, A. C. 1123  
 Rosa, F. 122  
 Rosen, J. B. 1236  
 Rosenbauer, J. 314, 322, 897, 964  
 Rosenblum, J. S. 111  
 Rosengård-Bärlund, M. 1176  
 Rosengren, V. 519  
 Rosenstock, J. 3, 6, 109, 202, 581, 764, 807, 840, 916, 917, 918, 919  
 Rosqvist, F. 78  
 Ross†, I. S. 1098  
 Rossi, M. C. 249, 1020  
 Rossignol, D. P. 742  
 Rossing, P. 1102, 1130, 1132, 1146, 1195, 1223, 1244  
 Rossmeisl, M. 642  
 Rössner, S. K. 696  
 Rostoka, E. 1208  
 Rosu, M. 900, 903  
 Roubalova, J. 642  
 Round, R. A. 984  
 Roura, E. 933  
 Rousev, R. G. 1084  
 Rousseau, M. F. 1268  
 Roussel, R. 80, 153, 300  
 Roy, A. 193  
 Roy, S. 190, 1120  
 Roze, S. 1041  
 Ruan, X. 120, 819  
 Rubio-Martin, E. 672, 870  
 Ruck, R. 1235  
 Ruckes, C. 974  
 Rudovich, N. N. 707  
 Ruessmann, H.-J. 1036  
 Ruetten, H. 811  
 Ruff, D. 772, 775  
 Ruggles, J. 799  
 Rugina, R. 607  
 Ruige, J. B. 654, 1219  
 Rukh, G. 288  
 Russell, M. A. 492  
 Russell-Jones, D. L. 38, 800, 910, 911, 995  
 Russell-Jones, E. C. 1023  
 Russo, E. 1196  
 Russo, I. 232  
 Rustan, A. C. 29  
 Rustenbeck, I. 399, 411, 413, 490  
 Rustico, C. 901  
 Rusu, E. D. 877  
 Rusu, F. 877  
 Rutkowska, M. 928, 934  
 Rutten, G. E. H. M. 868, 1022  
 Rütten, H. 809  
 Rutter, G. A. 101, 212, 526  
 Rutti, S. 389  
 Ruus, P. 808, 813  
 Ruzo, A. 447  
 Ryder, J. 953, 1005, 1035  
 Ryder, R. E. J. 801  
 Ryhänen, S. 266  
 S  
 Saab, C. 1070  
 Saad, A. 1056  
 Saad, F. 888  
 Saad, M. 555  
 Saada, A. 303, 304  
 Saatov, T. 1136  
 Sabater, M. 658  
 Sabau, S. 342  
 Saberi, M. 587  
 Sabia, H. D. 742  
 Sachon, C. 1091  
 Sacramento, J. F. 93, 554  
 Sadasivan Pillai, P. 360  
 Saeki, A. 783  
 Saely, C. H. 339  
 Säemann, M. D. 1138  
 Saenko, Y. 1173  
 Saga, N. 318  
 Sagarra, E. 1076  
 Sagarra, R. 352  
 Sage, D. 259  
 Sägerorp, J. 71  
 Saisho, Y. 462  
 Saito, A. 335  
 Saito, K. 313, 866, 1073, 1217  
 Saito, T. 767  
 Saji, H. 718  
 Sajid, W. 218  
 Sakagami, H. 578  
 Sakaguchi, K. 816  
 Sakai, P. 9  
 Sakai, S. 740, 741  
 Sakamoto, M. 844, 884  
 Sakoda, H. 734  
 Saksida, T. 497, 647  
 Sakuma, Y. 785  
 Sakuramoto-Tsuchida, S. 525  
 Sala, R. 1044  
 Salandini, S. 710  
 Saldalamacchia, G. 586  
 Saleem, M. A. 1125  
 Salles, J. 9  
 Salminen, P. 8, 565  
 Salmon, P. 259  
 Salonen, K. M. 266  
 Salsali, A. 744, 746, 750, 754, 755  
 Salunkhe, V. A. 486  
 Salvadori, R. 1166  
 Salvatoni, A. 90  
 Salvemini, L. 136  
 Salvucci, M. 412  
 Salzsieder, E. 1047  
 Sameshima, H. 779  
 Sammeth, M. 511  
 Sampaolo, L. 309  
 Sampol, G. 1267  
 Samuel, V. T. 19  
 Samuelsson, U. 278  
 Samukawa, Y. 740, 741, 742  
 Sanchez, R. 605  
 Sandelin, A. 1176  
 Sanders, T. 463  
 Sandholm, N. K. A. 170, 188  
 Sandre-Banon, D. 1094  
 Sands, A. 772, 773, 774, 775, 776  
 Sanfeliciano, S. G. 819  
 Sangalli, E. 122, 123  
 Sanguinetti, R. 575  
 Sankar, A. 45, 1154, 1181  
 Sannino, C. 1093  
 Sano, H. 348  
 Sano, H. 783  
 Santamaria, B. 94, 1125  
 Santamaria, P. 451  
 Santana, A. 328  
 Santos, G. J. 386  
 Santos, W. 58, 59  
 Sanz, C. 552  
 Sanz, R. 634  
 Sanz Nogues, C. 1263  
 Sapin, H. 5, 781  
 Saraheimo, M. 154, 170, 1135, 1137  
 Sarantopoulou, V. 1063  
 Saraste, A. 1261  
 Saravanan, P. 1017  
 Sardinian Group for Epidemiology of Type 1 Diabetes 324  
 Sargeant, L. A. 183  
 Sari, R. 940  
 Sarika, L. 1063  
 Saris, W. H. M. 709  
 Sarkady, D. 1152  
 Sartorius, T. 685  
 Saryusz-Wolska, M. 928, 931  
 Sasai, T. 1100  
 Sasaki, H. 1147  
 Sasaki, K. 179  
 Sasaki, T. 589  
 Sasaki, T. 740, 741  
 Sasson, S. 504  
 Sastre, J. 833  
 Sathananthan, M. 585  
 Satman, I. 40, 996  
 Sato, A. 1217  
 Sato, A. 319  
 Sato, C. 62  
 Sato, F. 426  
 Sato, J. 1107  
 Sato, S. 698  
 Sato, T. 426  
 Sato, Y. 469  
 Sato, Y. 503, 1257  
 Sato, Y. 648  
 Sato, Y. 741  
 Sato, Y. 831  
 Satoh, J. 1100  
 Saudek, F. 435, 1029  
 Saukkonen, T. 616  
 Saulnier, P.-J. 1122  
 Sauque Reyna, L. 807  
 Savolainen, A. M. 601  
 Savona-Ventura, C. 1070  
 Savu, O. 607  
 Sawada, S. 143  
 Sawbridge, L. 591  
 Sawicki, P. T. 961  
 Sbraccia, P. 643  
 Scaramuzza, A. 1058  
 Scarcia, P. 523  
 Scatena, F. 461  
 Scavini, M. 198, 1034  
 Scelza, N. 150  
 Schachner, H. C. 993  
 Schacht, A. 48  
 Schaefer, J. 1030  
 Schäfer, H.-L. 809  
 Schalkwijk, C. G. 61, 133, 682, 1239, 1255  
 Schall, T. J. 31  
 Schaller, H.-E. 683  
 Schaper, F. 199  
 Schauer, D. 979  
 Scheeler, M. 299  
 Scheen, A. J. 202  
 Scheerer, M. F. 635  
 Scheijen, J. 133  
 Scherbaum, W. A. 384  
 Schermann, M. 624  
 Scherneck, S. 673  
 Schernthaner, G. 5, 134, 145, 243, 624, 723, 727, 781, 850  
 Schernthaner, G.-H. 134, 624, 723, 727  
 Schiavon, C. A. 9  
 Schick, F. 299  
 Schiel, R. 1098  
 Schiess, S. 548, 550  
 Schiller, M. 654  
 Schimmenti, A. 137  
 Schinner, S. 384  
 Schiöler, L. 51  
 Schirra, J. 736  
 Schlag, A. 707  
 Schleicher, E. 542, 631, 685  
 Schleinitz, D. 680, 694  
 Schmeißer, S. 590  
 Schmidt, W. 1043  
 Schmitt, H. 202  
 Schmitter, J.-M. 397  
 Schmitz-Peiffer, C. 21  
 Schmoll, D. 548  
 Schneider, M. 1006, 1007  
 Schober, E. 898, 1066  
 Schoemaker, M. 1043  
 Schoene, K. 374  
 Schoeppe, T. 449  
 Schoonheim, M. M. 167  
 Schrauwen, P. 598  
 Schroeder, E. 353  
 Schroer, S. A. 205, 206, 510  
 Schroner, Z. 298  
 Schuiki, I. 518  
 Schulteis, C. 4, 799  
 Schultz, J. 98, 595  
 Schultz, N. 561  
 Schulz-Raffelt, G. 382  
 Schumacher, K. 399, 413, 490  
 Schumann, D. 398  
 Schumm-Draeger, P.-M. 926  
 Schupp, M. 210  
 Schürmann, A. 597, 673  
 Schuster, S. 499  
 Schwab, D. 767  
 Schwab, K. O. 314  
 Schwartz, A. V. 674

- Schwartz, S. 846, 980  
 Schwarz, P. E. H. 966, 1156  
 Schweitzer, M. 982, 1005, 1035  
 Schweizer, A. 862  
 Schwitzgebel, V. M. 491  
 Scobie, I. N. 1023  
 Scott, F. 446  
 Scott, M. 37  
 Scottish Diabetes Research Network Epidemiology Group 187, 233, 239, 1202  
 Scottish Diabetic Retinopathy Screening Collaborative 187  
 Scotton, R. 196, 968  
 Seabra, D. 1096  
 Sebastiani, G. 104, 431, 468  
 Secchi, M. 232  
 Secchiero, P. 646  
 Secher, A. L. 83  
 Sechterberger, M. K. 184  
 Seck, T. 36  
 Seckar, P. 879  
 Seferovic, J. 475  
 Seferovic-Mitrovic, J. 180  
 Segel, S. 909  
 Seghieri, G. 702, 1166  
 Sehgal, A. 628  
 Seino, H. 319  
 Seino, S. 421, 506, 816  
 Seino, Y. 219, 740, 741, 885, 966  
 Sejling, A.-S. 138, 221, 279  
 Sekerija, M. 251  
 Sekler, I. 101  
 Selbach, M. 213  
 Sell, H. 654  
 Selvarajah, D. 45, 1154, 1181  
 Selvaraju, R. 261  
 Semenova, D. A. 1185  
 Semiz, S. 630  
 Semplici, F. 101  
 Sen-Gupta, P. 801  
 Senba, E. 63, 686  
 Sendela, J. 970  
 Sener, A. 77, 485  
 Senges, J. 60  
 Senmaru, T. 258, 568  
 Senokuchi, T. 661  
 Seo, C. 844, 884, 950  
 Seo, M. 372  
 Séquaris, G. F. B. 22, 129, 131, 445  
 Serban, V. 900, 903  
 Sereno, J. 557  
 Serlie, M. J. 644  
 Serné, E. H. 609  
 Serra, E. 164  
 Serradas, P. 414  
 Serrano, M. 208, 295, 562, 658, 676  
 Serrano, S. 164  
 Serup, A. K. 588  
 Servitja, J. M. 121, 144, 540  
 Sesti, G. 2  
 Seubert, J. 52  
 Seufert, J. 507, 802, 1081  
 Sevdalis, M. 56  
 Seyfritz, E. 23  
 SFD-SFGG Intergroup 1106  
 Sgarbossa, A. 470  
 Sha, S. 244, 760  
 Shaat, N. 1074  
 Shadrach, J. 175  
 Shah, N. 1019  
 Shah, P. 162  
 Shah, S. 602  
 Shah, S. 940  
 Shahjahan, M. 1025  
 Shamanna, P. 732  
 Shamansurova, Z. 1136  
 Shan, S.-O. 338  
 Shankar, A. 360  
 Shankar, R. 834  
 Shao, Q. 857, 860  
 Sharipova, J. 1208  
 Sharkovska, Y. 35  
 Sharma, A. M. 696  
 Sharoyko, V. 97, 98, 99  
 Sharp, S. J. 183  
 Shaughnessy, L. 47  
 Shaw, G. 1172  
 Shaw, W. 764  
 Shcherbina, L. 176, 574  
 Shehata, A. M. 459  
 Sheikh, A. R. A. 370, 377  
 Shen, C. 1222  
 Shen, J. 817  
 Shen, Y. 349  
 Shen, Y. 872  
 Shepherd, M. H. 463  
 Sheppard, M. 358  
 Sheriff, M. H. R. 1097  
 Shestakova, M. 1148  
 Shetty, R. 732  
 Sheu, Y. 674  
 Shevalye, H. 1121, 1174  
 Shi, C. 112, 954  
 Shi, L. 1075  
 Shi, R. 719, 720  
 Shi, S. Y. 205, 206  
 Shi, Y. 483  
 Shibue, K. 179  
 Shields, B. M. 332, 371, 463  
 Shiga, T. 739  
 Shigeto, M. 70  
 Shikata, K. 62, 1115  
 Shimada, A. 171  
 Shimamoto, K. 111  
 Shimano, H. 430, 1217  
 Shimasaki, K. 742  
 Shimizu, M. 1108  
 Shimizu, S. 219, 533  
 Shimomura, I. 282, 423, 469  
 Shimpuku, M. 589  
 Shin, J. 627  
 Shirakawa, J. 705  
 Shiu, S. W. M. 1220  
 Shivane, V. K. 851, 852  
 Shlyachova, N. V. 466  
 Shojima, N. 734  
 Shojima, S. 973  
 Shore, C. R. 821  
 Shrivastav, A. 873  
 Shuldiner, A. R. 284  
 Shulman, G. I. 19, 559  
 Shusterman, A. 119  
 Shymanskyi, I. 450  
 Sica, M. 410  
 Sicari, R. 692  
 SID-CINECA “ARNO-Diabetes” Working Group 346  
 Sidney, S. 979  
 Siegel-Axel, D. I. 683  
 Sienkiewicz, A. 871  
 Siesky, A. M. 42, 819  
 Sigrist, S. 23  
 Sillars, B. A. 1200  
 Silva, A. S. 604  
 Silva, J. 58, 59  
 Silva, M. A. 1182  
 Silva, M. A. 292  
 Silva, M. G. 604  
 Silver, H. J. 939  
 Silver, K. D. 284  
 Sima, A. 900, 903  
 Sima, I. 877  
 Simell, O. 240, 265, 326, 473  
 Simmons, R. K. 183  
 Simó, O. 991  
 Simó, R. 5, 127, 781, 1112, 1117, 1267  
 Simon, C. 1033  
 Simonsen, R. 156  
 Simpson, P. 1051  
 Simpson, R. W. 780  
 Simpson, S. H. 52  
 Simpson, S. J. S. 432  
 Simrén, M. 46  
 Singh, G. 628  
 Sinha, V. P. 24, 913, 914, 916, 917, 922  
 Sinnott, M. 364  
 Siriwardena, A. N. 621  
 Sironi, A. 692  
 Sisino, G. 81  
 Sivappriyan, S. 1089  
 Sivasubramaniyam, S. 1027  
 Sivasubramaniyam, T. S. 206  
 Sixta, B. 255  
 Sjakste, N. 1208  
 Sjöholm, Å. 519, 535  
 Sjöström, C. D. 748, 751  
 Skarbaliene, J. 823  
 Skibinska, J. 1086  
 Skibová, J. 255, 256, 435, 1152  
 Skinner, T. C. 963  
 Skjoth, T. V. 40, 920  
 Skljarevski, V. 48  
 Skolnik, E. 1119  
 Skovlund, S. E. 1015  
 Škrha, J. 200  
 Škrha, J. Jr. 200  
 Skupien, J. 1068  
 Skurk, T. 717  
 Skyler, J. S. 41  
 Slagboom, P. E. 285  
 Slater, J. 837, 838  
 Sleddering, M. A. 731  
 Slenter, J. 1255  
 Sletner, L. 882, 1071  
 Slim, I. 330  
 Sloth, B. 709  
 Small, P. K. 7  
 Smillie, S. J. 1209  
 Smirnova, O. M. 465  
 Smit, J. W. A. 1219  
 Smith, D. 1030  
 Smith, D. 521  
 Smith, H. T. 373  
 Smith, M. 774, 776  
 Smith, M. S. 24  
 Smith, N. 839  
 Smith, S. 1019  
 Sneed, W. 224  
 Snoek, F. J. 167  
 Soare, A. 115  
 Sobngwi, E. 1013  
 Soewondo, P. 951  
 Søfteland, E. 46  
 Sogayar, M. C. 404  
 Sohn, E. 1111  
 Soinio, M. 565  
 Sokolova, L. K. 691  
 Sokolovska, J. 1208  
 Solà-Morales, O. 352  
 Solimena, M. 331, 398, 410  
 Solini, A. 1139  
 Solis-Herrera, C. 245  
 Solomon, M. 619  
 Solomon, T. P. 600  
 Someya, Y. 318, 865  
 Somm, D. 1021  
 Somm, E. 491  
 Sommer, C. 882  
 Søndergaard, F. 924  
 Sone, H. 313, 319, 866, 1073, 1101, 1197, 1217, 1243  
 Sonestedt, E. 288, 290, 869  
 Song, E. 787, 1228, 1230  
 Song, S. 378  
 Song, X. 1254  
 Songini, M. 324  
 Sönmez, A. 331  
 Sonne, D. P. 573  
 Soon, D. K. W. 914  
 Sørensen, J. 261  
 Sørgerd, E. P. 85  
 Soriguer, F. 672, 870  
 Sorli, C. 9  
 Sosa, L. 410  
 Sosale, A. R. 851, 852  
 Soty, M. 121, 539  
 Souhami, E. 807  
 SOUL-D Research Group 359  
 Šoupal, J. 200  
 Sourris, K. C. 151  
 Sousa, H. 1169  
 Sousa, P. 58, 59  
 Souverein, P. C. 354  
 Sozu, T. 363  
 Spégel, P. 97  
 Spadafora, L. 137  
 Spagnuolo, I. 104, 431, 468, 1085  
 Spallone, V. 1180  
 Spalova, I. 476  
 Spanheimer, R. 787, 1228  
 Spanou, L. 1080  
 Spanoudi, F. 1240  
 Späth, M. 48  
 Spauwen, P. J. J. 18  
 Spigoni, V. 124  
 Spinas, G. A. 388  
 Spinetti, G. 122, 123  
 Spinnler, R. 499  
 Spital, G. 193  
 Spoletini, M. 269, 896  
 Spranger, J. 694  
 Spreghini, M. R. 901  
 Sramkova, P. 726  
 Sreenan, S. 626  
 Srinivasan, A. 1037  
 Srinivasan, B. T. 311  
 Srinivasan, P. 441  
 Stachow, R. 314  
 Stachs, O. 43, 44  
 Stadlbauer, K. 411  
 Stahl, A. 322, 897, 964  
 Stahn, A. 231  
 Staiger, H. 299  
 Stančáková, A. 284  
 Stanarcic, J. 180  
 Stappenbeck, N. 162  
 Starkstein, S. E. 250  
 START Study Team 454  
 Stathi, C. 366, 867  
 Staudte, R. 199  
 Stausbol-Gron, B. 13  
 Stavniichuk, R. 1121

- Stechl, J. 813, 814  
 Stechova, K. 476  
 Stefan, N. 299, 683  
 Stefanadis, C. 1129  
 Stefanczyk, L. 928  
 Stefanini, E. 1225  
 Stefanova, E. 180  
 Stehouwer, C. D. A. 18, 61, 133, 234, 682, 1239, 1255  
 Steiner, J. 199  
 Steiner, J. F. 353  
 Steinsträßer, A. 808  
 Stene, L. C. 88  
 Stenger, P. 993  
 Stenlöf, K. 760, 765  
 Stepanova, E. N. 472  
 Stepanova, S. M. 1185  
 Steven, S. 7  
 Stevens, M. 1270  
 Stevens, R. J. 788, 789  
 Stewart, J. 935, 944  
 Stewart, M. 581  
 Sticht, C. 707  
 Stienstra, R. 66, 560, 659  
 Stock, J. 895  
 Stock, U. A. 683  
 Stöckl, D. 1161  
 Stoffel, M. 486  
 Stoica, V. 877  
 Stojanovic, I. 497, 647  
 Stojkovic, I. 288  
 Stolz, K. 499, 501  
 Storgaard, H. 1264  
 Störing, J. 281  
 Storme, L. 81  
 Storrs, R. 264, 439  
 Stosic-Grujicic, S. 497, 647  
 Stottrup, C. 1120  
 Strassburger, K. 103, 294, 310, 322, 964  
 Štrbák, V. 438  
 Streeffland, T. C. M. 611  
 Streit, J. 839  
 Strele, I. 699  
 Stroll, B. 1001  
 Strøm, M. S. 625  
 Strom, T. 147  
 Strongin, L. G. 15, 230, 941  
 Strozik, A. 657  
 Struchkova, J. 230  
 Struck, J. 135, 155, 345  
 Stuart, W. 422  
 Stulnig, T. M. 880  
 Stumvoll, M. 680, 694  
 Sturis, J. 915  
 Su, G. 229  
 Subramaniam, G. 793  
 Sudar, Z. 1092  
 Sue, N. 515  
 Suen, C. S. 819  
 Suesca, E. 1169  
 Sugawara, A. 1073  
 Sugawara, K. 219, 885  
 Sugg, J. E. 242, 721, 743, 745, 748, 751, 753, 755, 756, 758, 777  
 Sugihara, H. 503, 1257  
 Sugino, M. 783  
 Sugiri, D. 307  
 Sugisawa, E. 165  
 Sugizaki, K. 219  
 Sugoka, O. 1208  
 Suhre, K. 635  
 Suleiman, M. 437, 461, 493  
 Sullivan, D. 762, 765  
 Sullivan, T. J. 31  
 Sultan, A. 55, 1199, 1218  
 Sultan, S. A. A. 1259  
 Sultana, S. 1078  
 Sultana, S. S. 1232  
 Summanen, P. 188  
 Summers, N. 619  
 Sun, F. 1009  
 Sun, X. 688  
 Sun, Z. 365, 537, 994, 1113, 1145,  
 Sund, R. 237  
 Sundström, J. 690  
 Sung, Y. 1177  
 Sunkari, V. G. 1213, 1214  
 Sunyer, B. 352  
 SUPREME-DM Study Group 353  
 Surendan, P. 65  
 Sury, M. 213  
 Suryawanshi, S. 110  
 Suzuki, H. 430, 1217  
 Suzuki, J. 698  
 Suzuki, K. 179  
 Suzuki, M. 858  
 Suzuki, R. 734  
 Suzuki, S. 319  
 Suzuki, S. 831  
 Suzuki, Y. 785  
 Svalgaard, J. 481  
 Sveen, K. A. 1162  
 Svendsen, A. M. 218  
 Svendsen, B. 409  
 Svendsen, L. B. 248, 582  
 Svennblad, B. 690  
 Svensson, A.-M. 51, 182  
 Svensson, J. 1213  
 Svensson, J. 276, 614  
 Svensson, M. K. 549  
 Svojanovsky, J. 152, 1128  
 Swift, P. 238, 963  
 Syed, F. 437, 461, 493  
 Sykova, E. 255  
 Szabo, M. I. 1010  
 Szadkowska, A. 1237  
 Szamatowicz, J. 1086  
 Szczepaniak, E. 794  
 Szczepaniak, L. S. 794  
 Szczepura, A. 1017  
 Szendrödi, J. 22  
 Szendroedi, J. 129, 131, 559  
 Szewczyk, L. 899, 904  
 Sziártó, I. 1144  
 Szopa, M. 333, 1068  
 Szymanska-Garbacz, E. 928, 931  
 Szybowska, A. 970, 1053  
 T  
 't Hart, L. M. 285  
 T1DGC 328  
 Tabák, A. G. 356, 357, 1092  
 Tabarkiewicz, J. 899  
 Tack, C. J. 66, 560, 659, 929, 942  
 Tada-Iida, K. 430  
 Taes, Y. E. 654  
 Tafuro, S. 447  
 Taguchi, K. 589, 927  
 Tahrani, A. 222, 1270  
 Tajima, K. 705  
 Tajima, N. 348  
 Tajiri, Y. 257  
 Takabe, M. 816  
 Takács, R. 1158  
 Takagi, M. 718  
 Takahashi, A. 1217  
 Takahashi, H. 421  
 Takahashi, I. 426  
 Takahashi, K. 171, 1100  
 Takahashi, M. 553  
 Takahashi, N. 927  
 Takahashi, S. 698  
 Takahashi, T. 1100  
 Takahiro, I. 1124  
 Takaki, Y. 661  
 Takano, Y. 768  
 Takao, T. 376  
 Takasawa, S. 525  
 Takatsuka, T. 62, 1115  
 Takbou, K. 116  
 Takebe, N. 1100  
 Takeda, J. 640  
 Takei, M. 831  
 Takemoto, M. 1124, 1197, 1212  
 Takiyama, Y. 578  
 Takizawa, M. 335, 337  
 Takkinen, H.-M. 265, 326  
 Talbot, N. A. 591, 668  
 Talchai, C. 141  
 Talton, J. 1238  
 Talukdar, S. 541  
 Tamagawa, Y. 973  
 Tamaki, S. 525  
 Tamarit-Rodriguez, J. 406  
 Tamas, G. 1092  
 Tamayo, T. 307  
 Tamazouzt, A. 72  
 Tamburello, A. 782  
 Tamer, S. C. 117, 910, 911  
 Tan, K. C. B. 1220  
 Tan, R. 1116  
 Tan, S. 91  
 Tanaka, H. 460  
 Tanaka, M. 63  
 Tanaka, S. 319  
 Tanaka, S. 319, 1243  
 Tanaka, S. 171  
 Tanaka, Y. 1073  
 Tanaka, Y. 1108  
 Tanaka, Y. 301  
 Tanaka, Y. 742  
 Taneichi, H. 1100  
 Tang, A. 494  
 Tang, H. 441  
 Tang, J. 872  
 Tang, X. 368  
 Tangirala, M. 936  
 Tanhauserova, V. 152, 1128  
 Taniguchi, S. 401  
 Tankiewicz, S. 1030  
 Tanoue, E. 779  
 Tao, H. 229  
 Tapager, I. 976  
 Tapia, G. 88  
 Tarasov, A. I. 101  
 Tarasov, S. A. 887  
 Tarkia, M. 1261  
 Tarnow, L. 47, 620, 1130, 1146, 1159, 1160, 1195, 1245, 1264  
 Taroni, S. 1165  
 Tartaglia, A. 1165  
 Tashmanova, A. 1136  
 Taskinen, M.-R. 327, 853  
 Tasneem, A. 204  
 Taton, J. 17  
 Tatsuoaka, H. 733  
 Tattikota, S. 213  
 Tauber, S. 671  
 Tauscher, R. 678  
 Tauschmann, M. 453  
 Tauveron, I. 1003  
 Tavares, I. 1182  
 Tay, D. 361  
 Tay, T. 1187  
 Taylor, A. 843  
 Taylor, R. 7  
 Tchankou, C. 178  
 Tedeschi, A. 1166  
 Teede, H. J. 1105  
 Teichenne, J. 418  
 Teige, M. 231  
 Teixeira, P. C. 404  
 Telejko, B. 1086  
 Tellez, N. 427  
 Tengholm, A. 68, 69, 71, 102, 383  
 Tennagels, N. 544, 706  
 Tenoutasse, S. 267  
 Tentolouris, N. 369, 724, 1109, 1129, 1241  
 Teodoro, T. 518  
 Teräs, M. 1261  
 Terasaki, M. 778, 786  
 Terauchi, Y. 705, 864  
 Terra, L. F. 404  
 Teruyama, K. 506  
 Tesfaye, S. 45, 48, 1154, 1181  
 Tesi, F. 702  
 Teulon, J. 414  
 Tezyaeva, S. A. 941  
 Thaler, J. 727  
 Thankamony, A. 96  
 Thannberger, P. 1269  
 Thanopoulou, A. 1070  
 Theilade, S. 1132, 1146, 1244  
 Theis, F. J. 169  
 Theodoraki, A. 392  
 Thiel, S. 1195  
 Thiemann, S. 6  
 Thiering, E. 895  
 Thiviolet, C. 532  
 Thomas, A. 231  
 Thomas, D. 1263  
 Thomas, H. E. 531  
 Thomas, L. 1181  
 Thomas, M. C. 646  
 Thomas, R. L. 1142  
 Thomsen, A. B. 802  
 Thomsen, H. F. 925  
 Thong, K. Y. 801  
 Thorand, B. 103, 294, 308  
 Thorén Fischer, A.-H. 207  
 Thorens, B. 70  
 Thoresen, H. 29  
 Thorn, L. M. 154, 170, 1135  
 Thorsted, B. L. 626  
 Thorsteinsson, B. 138, 221, 279, 620  
 Thrane, M. 923  
 Thrysoe, S. 13  
 Thuan, J. 1265  
 Tian, G. 71  
 Tian, H. 587  
 Tian, H. 835, 836  
 Tian, L. 229  
 Tiberti, C. 104  
 Tiedge, M. 43, 98, 128, 449, 595, 596  
 Tien, T. 190  
 Tiengo, A. 1034  
 Tiitu, A. 1176  
 Tikellis, C. 646  
 Tilak, P. 793  
 Tile, S. 463  
 Till, H. 678



- Timar, B. 900, 903  
 Tinahones Madueño, F. J. 293, 295  
 Tippu, Z. 385  
 Tirado, R. 725  
 Tiu, C. 342  
 Tkac, I. 298  
 Tobe, K. 301  
 Tobin-Hess, A. 706  
 Tochlin, A. 788  
 Todd, M. 1064  
 Todorova - Ananieva, K. N. 1084  
 Toffanello, G. 343  
 Togashi, Y. 705  
 Tokmakova, A. U. 1185  
 Tokunaga, H. 973  
 Tolborg, J. L. 177, 823  
 Tölle, T. 48  
 Tomcalova, J. 879  
 Tomita, R. 640  
 Tomoyasu, M. 786  
 Tong, C. 760  
 Toniolo, A. 90  
 Tönjes, A. 680, 694  
 Tønnesen, E. 95  
 Töns, H. A. 433  
 Töpfer, E. 331  
 Topp, B. G. 914  
 Torjesen, P. A. 148  
 Torkko, J. M. 410  
 Tornoczky, J. 1092  
 Toropova, O. K. 458  
 Torre, M. 720  
 Torrejón, R. 1061  
 Torrens, D. 295  
 Torres, A. 1179  
 Tortosa, F. 513, 1260  
 Tory, K. 1248  
 Toubro, S. 762  
 Toumaniantz, G. 553  
 Tousoulis, D. 1129  
 Towae, F. 60  
 Townsend, R. 746  
 Toyoda, K. 718  
 Toyoshima, H. 430  
 Trab Damsgaard, M. 963  
 Tracz, M. 940  
 Tragni, E. 945  
 Tran, A. 20  
 Trapp, S. 716  
 Trautmann, M. 5  
 Trautsolt, W. 657  
 Travert, F. 80  
 Trefely, S. 91  
 Trevisan, R. 1139  
 Triadafilopoulos, G. 628  
 Triantafyllidi, H. 1240  
 Tricarico, M. 1133  
 Tripathy, D. 563, 564, 791  
 Triplitt, C. 245  
 Trippenbach-Dulska, H. 970, 1053  
 Triscari, J. 1235, 1236  
 Trischitta, V. 136, 1196  
 Tritakis, V. 1240  
 Trombetta, P. 198  
 Tronko, M. D. 458  
 Tronko, N. D. 691  
 Troupin, B. 696  
 Trovati, M. 232, 701  
 Tryggvason, K. 170, 1124, 1212  
 Tsapas, A. 241  
 Tsaroucha, E. 507  
 Tsatsoulis, A. 474, 477  
 Tschank, G. 544  
 Tschöpe, D. 60  
 Tschopp, O. 388  
 Tsiakou, A. 366, 867, 1109  
 Tsotoulidis, S. 1157  
 Tsoy, A. 1136  
 Tsuchida, H. 640  
 Tsuchiya, K. 894  
 Tsuji, H. 313, 866  
 Tsuji, T. 885  
 Tsujinaka, H. 525  
 Tsujino, D. 844, 884, 950  
 Tsun, J. G. S. 1220  
 Tsunoda, T. 172  
 Tsuprykov, O. 35  
 Tu, Y. 73  
 Tudhope, S. 608  
 Tuduri, E. 570  
 Tuomi, T. 108, 273, 482  
 Tuomilehto, J. 340  
 Tuominen, L. 28  
 Turnage, A. 772, 773, 775  
 Turnbull, J. 392  
 Turner, N. 591, 638, 649  
 Tuthil, B. 995  
 Tulari, J. J. 28  
 Tuvia, N. 210  
 Tybjærg-Hansen, A. 344  
 Tymchysyn, O. 1030  
 Tzemos, K. 369  
 Tzirogiannis, K. 16, 56
- U**  
 Uchigata, Y. 165, 335, 337, 362  
 Uddin, A. 341  
 Ueda, H. 650  
 Ueda, T. 973  
 Ueki, K. 460, 734  
 Ueno, M. 698  
 Ueyama, E. 739  
 Ülgen, F. 384  
 Ulianich, L. 545  
 Ullich, S. 382, 685  
 Ulmannova, T. 476  
 Ulven, T. 382  
 Umematsu, H. 734  
 Umpleby, M. A. 195  
 UNITED Research Team 358  
 Ünütürk, U. 937  
 Uno, S. 423, 469  
 Uno, Y. 589  
 Unser, M. 259  
 Urbano, F. 577  
 Urbina, E. 1238  
 Urheim, S. 1226  
 Urschitz, M. 924  
 Ursli, M. 134  
 Uruska, A. 163  
 Usberti, E. 124  
 Usiskin, K. 759, 762, 765  
 Usui, R. 219, 885  
 Usui, T. 589  
 Utsuno, A. 739  
 Utsunomiya, K. 348, 844, 884, 950  
 Uusitalo, L. 265, 326  
 Uysal, A. R. 937
- V**  
 Vaag, A. 96, 297, 558, 561, 592, 599, 1245  
 Vaccheris, C. 701  
 Vaillant, F. 424  
 Vaittinen, M. 722  
 Valadares, W. P. 604  
 Valcárcel, J. 286  
 Valdés, S. 870  
 Valdes, V. 336  
 Valeeva, F. V. 1062  
 Valensi, P. 32, 116, 1094, 1199, 1206, 1215  
 Valentini, A. 1191  
 Valentini, U. 968  
 Valentino, R. 651  
 Valladares, S. 1267  
 Vallarino, C. 704, 1229, 1231  
 Valle, M. 232, 701  
 Vallejo, M. 508  
 Valsamakis, G. 1082  
 Valverde, Á. M. 94, 1118, 1125  
 Vambergue, A. 81  
 van 't Riet, E. 234, 354, 988  
 van Asseldonk, E. J. P. 66, 560  
 van Boxtel, M. P. J. 18  
 van Can, J. 709  
 Van Coppenolle, F. 532  
 Van Dalem, A. 267  
 van der Heijden, M. M. P. 252  
 van der Kallen, C. 61, 133, 682, 1239  
 van der Meer, K. 1018  
 van der Meer, R. W. 1219  
 van der Meij, S. 65  
 van Diepen, J. A. 66  
 van Dijk, P. R. 932  
 van Dooren, F. E. P. 252  
 van Duinkerken, E. 167  
 Van Genugten, R. E. 609  
 van Golen, L. W. 687  
 van Greevenbroek, M. M. J. 61, 133, 682, 1239  
 van Haften, T. W. 285  
 van Hateren, K. J. J. 135, 345, 1018  
 van Leeuwen, N. 285  
 Van Nieuwenhove, Y. 654  
 van Oudenaren, A. 656  
 van Poppel, P. C. 560  
 Van Raalte, D. H. 609  
 Van Troostenburg de Bruyn, A.-R. 787, 1228  
 van Vuure, K. H. 1022  
 van Wagenveld, B. 65  
 van Zandvoort, M. A. M. 61  
 van Zonneveld, A. J. 656  
 Vandenplas, G. 1219  
 Vandoni, M. 1175  
 Vangen, S. 1071  
 Vania, A. 896  
 Vankova, M. 1194  
 Vantighem, M.-C. 434  
 Vardarli, I. 246  
 Vargha, P. 1092  
 Várkonyi, T. T. 1158  
 Varoudi, M. 1240  
 Varrault, A. 72  
 Vartholomatos, G. 474, 477  
 Vasconcelos, N.-M. 460  
 Vasileiou, V. 1063, 1080  
 Vassallo, J. 1070  
 Vassilakou, D. 241  
 Vatie, C. 178  
 Vaughan, E. E. 1172, 1263  
 Vaughn, D. E. 41, 907, 908  
 Vauthier-Brouze, D. 1091  
 Vavrova, E. 1029  
 Vazquez, C. 419  
 Vazquez-Montes, M. 789  
 Vcelak, J. 726, 1194  
 Vecsei, Z. 569  
 Vedovato, M. 1029  
 Veedfald, S. 248, 582  
 Vejola, R. 240, 265, 326, 473  
 Vejrazkova, D. 1194  
 Velho, G. 153, 300  
 Veliky, M. 450  
 Vella, A. 585  
 Vellinga, A. 79  
 Velloso, L. A. 556, 652  
 Veltman, D. J. 687  
 Vendelbo, M. H. 95  
 Venditti, C. 269, 896  
 Venesmaa, S. 722  
 Venugopalan, R. 1057  
 Venuti, R. 47  
 Verberne, H. J. 677  
 Vercruysse, F. 761, 764, 766  
 Vergès, B. 1224  
 Vergniol, J. 876  
 Verhey, F. R. J. 18  
 Verkauskiene, R. 1058  
 Vernea, F. 303  
 VERNY, C. 1106  
 Verras, C. 56  
 Vervoort, G. 942  
 Vespasiani, G. 57, 249  
 Vesper, I. 953, 1005, 1035  
 Vest, J. 822  
 Vestergaard, H. 1083  
 Vetrani, C. 878  
 Vexiau, P. 80  
 Vial, G. 532  
 Viberti, G. 793  
 Vicente, A. 833  
 Vick, A. 42  
 Vickers, S. P. 771  
 Vidal, H. 209, 667  
 Vidal, J. 12  
 Vidal, M. 479  
 Vidal, P. 1003  
 Vidal Puig, A. 209  
 Vides, H. 1236  
 Vieira, E. 394, 645  
 Vietz Andreassen, K. 114  
 Vigier Simorre, N. 1265  
 Vigili de Kreutzenberg, S. 693  
 Vigorito, C. 76  
 Vigouroux, C. 178  
 Vijan, S. 619  
 Vikman, J. 861  
 Vilaseca, M. 427  
 Vilches, A. 385  
 Vilches-Flores, A. 380  
 Vileikyte, L. 1184  
 Villacampa, P. 191  
 Villalpando, G. 1179  
 Villaplana, M. 725  
 Villars, C. 1033  
 Villena, J. A. 127, 1117, 1193  
 Vilsbøll, T. 248, 334, 572, 573, 582  
 Vinaixa, M. 684  
 Vincent-Tassin, E. 1122  
 Vintila, M. 607  
 Viretto, M. 232  
 Virreira, M. 485  
 Virtanen, K. 601  
 Virtanen, S. M. 265, 326  
 Visa, M. 121, 539, 540  
 VISS Study Group 1099  
 Vistisen, D. 356, 357, 370, 377  
 Vistoli, F. 150  
 Viswanathan, V. 793

- Vitagliano, G. 586  
 Vitai, M. 569  
 Vitale, M. 878  
 Vitale, V. 343  
 Viviani, G. L. 575  
 Vivot, A. 1246  
 Vlad, A. 903  
 Vogel, C. 953, 1005, 1035  
 Vogel, C. 898  
 Vogel, H. 597, 673  
 Vogt, L. 1047  
 Voigt, A. 26, 130  
 Volchuk, A. 518  
 Volkov, P. 105, 107, 142  
 Volpe, L. 1072  
 von Eynatten, M. 36, 848, 849, 850, 1233  
 von Meyer, A. 325  
 von Scholten, B. 1132  
 Vonbank, A. 339  
 Vora, J. 117  
 Voskanyan, G. 1054  
 Vourvou, M. 16  
 Vrbikova, J. 726  
 Vrieze, A. 65, 644  
 Vrolijk, H. 433  
 Vrtovec, M. 738  
 Vucic Lovrencic, M. 1150  
 Vujicic, M. 647  
 Vuori, N. 188  
 Vupputuri, S. 353
- W**
- Wada, N. 257  
 Wada, Y. 733  
 Wadén, J. 154, 170  
 Wadwa, R. P. 1238  
 Waeber, G. 391, 520  
 Wagenaar, A. 1255  
 Wagers, A. 175  
 Waget, A. 566  
 Wägner, A. M. 328, 1258  
 Wagner, B. 494  
 Wagner, I. 678  
 Wagner, K.-U. 205  
 Wagner, O. 671  
 Wagner, R. S. 953, 982, 1035  
 Wahren, J. 1110  
 Wailemann, R. A. M. 404  
 Wainstein, J. 119, 567, 1000  
 Wajs, E. 759  
 Wakasaki, H. 1147  
 Wake, D. 990  
 Walch, A. 664  
 Walczyk, J. 1068  
 Waldeck, B. 374  
 Waldeyer, R. 347  
 Wali, J. A. 531  
 Walker, J. 990  
 Walker, J. J. 233  
 Walker, M. 302, 306  
 Wall, D. 999  
 Wallensteen, M. 238  
 Waller, A. 990  
 Wallner, M. 1138  
 Walraven, I. 234  
 Walsh, C. 364  
 Walters, S. 1030  
 Wang, E. 938, 943  
 Wang, G.-S. 446  
 Wang, H. 507  
 Wang, H. 846  
 Wang, J. 1204  
 Wang, J. 174  
 Wang, J. 451  
 Wang, J. 527  
 Wang, L. 1009  
 Wang, L. 1009  
 Wang, L. 464  
 Wang, M. 856  
 Wang, N. 315  
 Wang, P. 948  
 Wang, R. 393, 416, 422  
 Wang, S. 1145  
 Wang, X. 1030  
 Wang, X. 30  
 Wang, X. 480  
 Wang, Y. 1113  
 Wang, Y. 1113  
 Wang, Y. 1220  
 Wang, Y. 1254  
 Wangnoo, S. K. 732  
 Wanic, K. 602  
 Wanke, R. 505  
 Ward, G. 1054  
 Wareham, N. J. 173, 183  
 Warin, J. 434  
 Wary, C. 1215  
 Waszczeniuk, M. 689  
 Watada, H. 301, 847, 865  
 Watanabe, H. 1197  
 Watanabe, K. 430  
 Watanabe, K. 219, 885  
 Watanabe, T. 740, 741  
 Watarai, A. 1247  
 Watcho, P. 1121, 1174  
 Waterstradt, R. 43, 98  
 Watkins, E. 925, 1042, 1046  
 Wawrusiewicz-Kurylonek, N. 689, 1086  
 Weaver, C. 799  
 Webb, D. 311  
 Webber, J. 1090  
 Weber, A. 1024  
 Weets, I. 267  
 Wegbrod, C. 331  
 Wei, L. 744, 754  
 Wei, W. 936, 986  
 Wei, X. 822  
 Weigert, C. 299, 542, 631, 685  
 Weir, G. C. 147  
 Weise, S. 678  
 Weiss, H. 43, 128, 449, 596  
 Weiß, J. 129  
 Weissmann, J. 1036  
 Weksler-Zangen, S. 303, 304  
 Welling, A. 487  
 Welschen, L. M. C. 354  
 Welsh, G. I. 1125  
 Welsh, J. B. 627, 1054, 1059  
 Welte, S. 544  
 Welungoda, I. 780  
 Wen, J. 1155  
 Wendt, A. 486  
 Weng, J. 349, 641, 835, 1048, 1114  
 Wens, J. 966  
 Wenzlau, J. M. 267  
 Werner, U. 706, 809, 811  
 Wessman, C. 744, 754  
 West, N. A. 106  
 Westenbroek, R. 483  
 Wetter, F. 520  
 Wettergren, A. 248, 582  
 Wetzels, K. 148  
 Wetzels, J. F. M. 1149  
 Weyerer, S. 997  
 Weykamp, C. 1149  
 Whaley, J. 752  
 Wheeler-Jones, C. P. 668  
 White, K. E. 1209  
 White, S. 264  
 Whitehead, J. P. 591  
 Whitehurst, T. K. 1030  
 Wichmann, H.-E. 103, 294  
 Wick, K. 952, 959  
 Wickham, B. 1077  
 Widdop, R. E. 780  
 Widmann, C. 516  
 Wiebe, J. C. 1258  
 Wiebe, J. C. 328  
 Wieland, T. 1119  
 Wierup, N. 11, 97, 176, 207, 574  
 Wierusz-Wysocka, B. 163, 227, 971  
 Wietrzycowska, R. S. 901  
 Wiinberg, N. 1245  
 Wijnands, E. 157  
 Wilbur, K. 547  
 Wild, S. 1202  
 Wilding, J. P. H. 721, 748, 751, 753, 766  
 Wilhelm, J. 1269  
 Wilhelm, S. 48  
 Wilinska, M. E. 195  
 Wilk, J. 689, 1086  
 Wilkinson, I. 45  
 Willaing, I. 960, 976  
 Wille, S. 940  
 Willemin, G. 20  
 Willenborg, M. 413, 490  
 Willi, S. M. 147, 454  
 Williams, B. 1146, 1244  
 Williams, K. M. 183  
 Williams, L. 1090  
 Williams, P. 37  
 Wilson, A. 1017  
 Wilson, B. 906  
 Wilson, C. 840, 841, 842  
 Wilson, L. 990  
 Windeløv, J. A. 713  
 winDiab. 855  
 Winding, K. 600  
 Winge, S. 218  
 Winkler, C. 169  
 Winkley, K. 359, 958  
 Winnick, J. J. 224  
 Winter, K. 44  
 Wintle, M. 804, 832  
 Winzell, M. S. 861  
 Wirfält, E. 288  
 Wirström, T. 893  
 Witek, P. 254  
 Witkowska, A. 657  
 Witso, E. 88  
 Witte, D. R. 356, 357, 370, 377, 700  
 Witte, N. 210  
 Wittmann, I. 1144  
 Wittmann, T. 1158  
 Wittrup, M. 226  
 Wium, C. 571  
 Wiza, C. 543  
 Wod, M. 271  
 Woerle, H.-J. 6, 36, 148, 770, 847, 848, 849, 850, 1233  
 Wohlfart, P. 811  
 Wojan, M. 678  
 Wojciechowska-Luźniak, A. 17  
 Wojtaszewski, J. 217  
 Wolden, M. L. 626  
 Wolden, M. L. 957, 969  
 Wolf, E. 505  
 Wolf, G. 952, 959, 998, 1098, 1205  
 Wolf, M. 924  
 Wolffenbittel, B. H. R. 201  
 Wolfram von Wolmar, C. 1006, 1007  
 Wollheim, C. B. 105, 507  
 Wong, M. 416  
 Wong, S. 456  
 Wong, Y. 1220  
 Woo, M. 205, 206, 510  
 Woo, V. 753, 940  
 Wood, I. 746  
 Woolf, A. S. 1209  
 Worm, D. 10, 588, 1245  
 Woskova, V. 255, 256  
 Wright, Jr., J. R. 451  
 Wu, E. 1227  
 Wu, L. 1251  
 Wu, L. 189  
 Wu, T. 247, 365, 820  
 Wu, X. 510, 1222  
 Wuchert, L. 595  
 Wudelehu, W. 640  
 Wudy, S. 316, 881  
 Wünsch, A. 505  
 Wuttke, A. 383  
 Wygant, G. 746, 755  
 Wystrychowski, G. 657
- X**
- Xi, L. 759  
 Xia, M. 73, 312, 375  
 Xia, X. 1114  
 Xiang, G. 946  
 Xiaohe, L. 715  
 Xie, B. 403  
 Xie, J. 762, 763  
 Xie, L. 986  
 Xie, Z. 365  
 Xing, X. 835  
 Xiong, Y. 338  
 Xu, B.-Y. 451  
 Xu, F. 641, 1114  
 Xu, G. 631  
 Xu, J. 368  
 Xu, J. 420  
 Xu, L. 834  
 Xu, M. 315  
 Xu, M. 1153  
 Xu, Q. 73  
 Xu, W. 1048  
 Xu, Y. 1009  
 Xu, Y. 1222  
 Xu, Y. 819
- Y**
- Yabe, D. 219, 885  
 Yachi, Y. 1073, 1243  
 Yada, T. 405, 488  
 Yale, J.-F. 759, 944  
 Yamada, K. 257  
 Yamada, N. 313  
 Yamada, N. 319, 430, 866, 1217  
 Yamada, T. 143  
 Yamada, T. 305  
 Yamada, T. 462  
 Yamaguchi, T. 219  
 Yamamoto, N. 783  
 Yamane, S. 179, 363, 648  
 Yamane, T. 785  
 Yamaoka, M. 401

- Yamasaki, H. 1147  
 Yamasaki, M. 111  
 Yamashita, H. 171  
 Yamato, S. 488  
 Yamauchi, A. 525  
 Yamauchi, M. 927  
 Yamauchi, T. 113, 172, 734  
 Yamazaki, M. 568  
 Yan, H.-M. 73, 375  
 Yan, J. 1048, 1114  
 Yan, K. 1124  
 Yan, X. S. 353  
 Yanagimachi, T. 578  
 Yanagisawa, H. 376  
 Yang, B. 105  
 Yang, B. 994, 1113, 1145  
 Yang, E.-J. 891  
 Yang, F. 581  
 Yang, G. 483  
 Yang, G. 580, 715  
 Yang, H. 229  
 Yang, H. 587  
 Yang, I. V. 106  
 Yang, J. 1143  
 Yang, J. 704, 1229, 1231  
 Yang, S.-N. 483  
 Yang, S. J. 851  
 Yang, W.-P. 752  
 Yang, X. 1048  
 Yang, Y. H. C. 536  
 Yanjun, J. 580  
 Yao, M. 749  
 Yap, F. Y. T. 151  
 Yasuda, S. 742  
 Yasuhara, M. 1073  
 Yato, S. 1217  
 Yderstraede, K. B. 271, 1160  
 Ye, G.-L. 773  
 Ye, J. 641  
 Ye, L. 260  
 Ye, Z. 173  
 Yee, J. 243, 760  
 Yeo, G. 622  
 Yilmaz, C. 996  
 Yin, H. 1113, 1145  
 Yin, J. M. 537  
 Yin, M. 480  
 Ying, L. 750  
 Yki-Järvinen, H. 6  
 Yokoo, T. 430  
 Yokote, K. 1124, 1197, 1212  
 Yokoyama, H. 1101  
 Yoneda, S. 423  
 Yoshida, M. 488  
 Yoshida, M. 783  
 Yoshida, S. 739  
 Yoshida, S. 783  
 Yoshida, S. 785  
 Yoshihara, T. 865  
 Yoshiike, N. 362  
 Yoshikawa, A. 282, 423, 469  
 Yoshimoto, K. 525  
 Yoshimura, T. 559  
 Yoshizawa, S. 313, 866, 1243  
 Young, B. 341  
 Yu, J. 480, 1113  
 Yu, J. 483  
 Yu, L. 1255  
 Yu, L. 483  
 Yu, M. 1153  
 Yu, S. 704, 1229, 1231  
 Yu, Y. 688  
 Yuan, Q. 527  
 Yuan, T. 197  
 Yuen, M. M. A. 1031  
 Yung, S. S. Y. 1220  
**Z**  
 Zabbara, A. 782  
 Zaccardi, F. 1221  
 Zacksenhaus, E. 510  
 Zagami, R. M. 577  
 Zahn, R. 60  
 Zain, M. 300  
 Zaitsev, S. V. 1253  
 Zaitseva, I. I. 1253  
 Zalaznick, J. 752  
 Zamaklar, M. 475  
 Zambrowicz, B. 772, 773, 774, 775, 776  
 Zampetti, S. 269, 896  
 Zandee, W. T. 731  
 Zannoni, S. 343  
 Zanone, M. M. 1163  
 Zapletalova, J. 879  
 Zarkogianni, K. 1080  
 Zarra, E. 57  
 Zarse, K. 590  
 Zatterale, F. 545  
 Zauli, G. 646  
 Zavaroni, I. 124  
 Zdravkovic, N. 497  
 Zdunczyk, B. M. 970  
 Zelanis, A. 404  
 Zemni, R. 330  
 Zeng, H. 632  
 Zeng, L. 368, 1048  
 Zenimaru, Y. 698  
 Zeppetbauer, M. 131  
 Zerbini, G. 1139  
 Zethelius, B. 51, 54, 182  
 Zeyda, M. 880  
 Zeymer, U. 60  
 Zhai, Q. 537  
 Zhan, L. 636  
 Zhang, C. 120  
 Zhang, D. 19, 559  
 Zhang, G. 1048  
 Zhang, H. 798  
 Zhang, J. 641  
 Zhang, L. 315, 1114  
 Zhang, L. 518  
 Zhang, L. 715  
 Zhang, P. 1204  
 Zhang, P. 587  
 Zhang, Q. 487  
 Zhang, Q. 803  
 Zhang, S. 1210  
 Zhang, Y. 485  
 Zhao, B. N. 31  
 Zhao, B. R. 247  
 Zhao, M. 441  
 Zhao, S. 774, 776  
 Zhao, W. G. 197  
 Zhao, Y. 1075  
 Zhao, Y. 1114  
 Zheng, H. 1105  
 Zheng, H. 229  
 Zheng, T. 872  
 Zheng, X. 1214  
 Zhenguo Yu, J. 451  
 Zhivov, A. 44  
 Zhou, J. 4  
 Zhou, K. 50  
 Zhou, M. 587  
 Zhou, M. 798  
 Zhou, R. 821, 935  
 Zhou, S. 986  
 Zhou, Y. 229  
 Zhou, Y. 365  
 Zhu, D. 835  
 Zhu, Y. 1048  
 Zhu, Z. 795  
 Zhuk, S. 707  
 Zidi, B. 875  
 Ziegler, A.-G. 87, 145, 169, 325, 895  
 Ziegler, D. 44, 1161  
 Ziegler, I. 44  
 Ziegler, R. 953, 982, 1005, 1035  
 Zielinska, A. 1086  
 Zien, K. 512  
 Zierhut, M. 825  
 Zietek, T. 717  
 Zijlstra, E. 1045  
 Zillich, F. 952, 959  
 Zinai, S. 370, 377  
 Zink, R. 547  
 Zinker, B. 752  
 Zinman, B. 38, 39, 615, 920  
 Zinsmeister, A. R. 585  
 Zipris, D. 457  
 Zisser, H. C. 1057, 1060  
 Zmysłowska, A. 1237  
 Zoetendal, E. G. 65, 644  
 Zoffmann, V. 1016  
 Zonenberg, A. 871  
 Zoppini, G. 1139  
 Zorzano, A. 658  
 Zouaoui, C. 875  
 Zoungas, S. 1105  
 Zoupas, C. 1109  
 Zozulinska-Ziolkiewicz, D. 163, 227, 971  
 Zschornack, E. 1043  
 Zuellig, R. A. 388  
 Zuffardi, O. 336  
 Zurawski, A. 1021



# European Association for the Study of Diabetes

## EASD Executive Committee

President:	A.J.M. Boulton, UK (retires 2014)
Vice-President:	S. Del Prato I (retires 2014)
Vice-President:	F. Bosch, ES (retires 2012)
Honorary Secretary:	M. Walker, UK (retires 2013)
Honorary Treasurer:	M. Roden DE (retires 2014)
Chairman of the Postgraduate Education Committee:	C. Tack, NL (retires 2013)
Editor-in-Chief, DIABETOLOGIA:	J. Zierath, SE (retires 2013)

**EASD Council comprises of the Officers above and the following members:**

### Term Expiring 2012

J.-M. Boavida, Portugal  
C.-G. Östenson, Sweden  
A. Pfeiffer, Germany  
N. Wareham, UK

### Term Expiring 2013

F. Beguinot, Italy  
J. Dekker, Netherlands  
F. Karpe, UK  
V. Urbanavicius, Lithuania

### Term Expiring 2014

K. Birkeland, Norway  
J. Bodansky, UK  
C. Levy-Marchal, France  
P. Nawroth, Germany

The Past President, U. Smith, and the Secretary of the Postgraduate Education Committee, L. Czupryniak, are members of the Council ex officio.

### HONORARY AUDITORS

P. Diem, Switzerland (retires 2014); L. M. Gardete-Correia, Portugal (retires 2014)

### HONORARY MEMBERS

G. Alberti, UK; D. Andreani, IT; J.-P. Assal, CH; E. Cerasi, IL; O.-B. Crofford, USA; A. Czyzyk, PL; T. Deckert, DK; P. Freychet, FR; H. Keen, UK; E. Kohner, UK; P. Lefèbvre, BE; R. Luft, SE; C. Lurie, USA; Carl Erik Mogensen, DK; J. Nerup, DK; L. Orci, CH; G.M. Reaven, USA; J. Pirart, BE; S. Rahbar, USA; J. Roth, USA; E. Shafir, IL; D. Steiner, USA; R. Unger, USA; E. von Wasielowski, DE; W. Waldhäusl, AT; C.B. Wollheim CH; Paul Zimmet, AU

### GOLD MEMBERS

Astra Zeneca, Mölndal, Sweden; Bayer HealthCare, Berlin, Germany; Boehringer Ingelheim GmbH, Ingelheim, Germany; Bristol-Myers Squibb, Uxbridge, UK; Eli Lilly & Co., Indiana, USA; Janssen Pharmaceutical NV, Beerse, Belgium; LifeScan, Inc., High Wycombe, UK; Merck & Co. Inc., New Jersey, USA; Novartis Pharma AG, Basel, Switzerland; Novo Nordisk A/S, Bagsvaerd, Denmark; Pfizer Inc. New York, USA; Sanofi, Paris, France; Takeda, London, UK

### SILVER MEMBERS

Abbott Diabetes Care, Berks, UK; Arkay Europe, Amstelveen, Netherlands; C8 MediSensors, Inc. San Jose, USA; Daiichi Sankyo Europe GmbH, Munich, Germany; Roche Diagnostics GmbH, Mannheim, Germany

### ASSOCIATE MEMBERS

77 Elektronika Ltd., Budapest, Hungary; A. Menarini Diagnostics, Grassano, Italy; Amylin Europe Ltd., San Diego, USA; Becton Dickinson Europe, Pont-de-Claix, France; Dexcom Inc. Lund, Sweden; Medtronic International, Tolochenaz, Switzerland; Merck Serono s.a. Geneva, Switzerland; Owen Mumford Ltd., Oxford, UK; Ypsomed GmbH, Sulzbach, Germany;

The EASD Executive Committee is also the Executive Committee of the European Foundation for the Study of Diabetes (EFSD).

**EASD/EFSD office : Rheindorfer Weg 3, 40591 Düsseldorf, Germany - [www.easd.org](http://www.easd.org)**